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PHOSPHATES IN THE SUGAR TOLERANCE TEST

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The literature on the relationship of phosphate to carbohydrate metabolism is now so very extensive that a survey of it would constitute a research in itself. The investigations of Hartman and Bollinger[3] and earlier, that of Harrop and Benedict,[2] and of Barenscheen,[1] are perhaps the most outstanding. It is a well established fact that phosphates are involved in carbohydrate metabolism and that the amount of phosphate in the blood changes during the assimilation of glucose. These changes in blood phosphate can be observed either after feeding glucose or after administration of insulin. It has been suggested that the most valuable aid in the differential diagnosis of various types of disease which involve carbohydrate metabolism is the curve of inorganic blood phosphate obtained after the administration of glucose. Hartmann and Bolliger state that "abnormalities in carbohydrate metabolism may be divided into seven groups by means of the blood phosphate curve." At present we are unable to support this contention.

This paper consists of a report of a study of 230 patients to whom we have administered 100 grams of glucose by mouth and observed the changes in blood phosphate, in the hope of determining whether or not these phosphate changes are of diagnostic value.

METHODS EMPLOYED

In this study the blood sugar estimations were made by the picric acid method[7] of Benedict, and inorganic blood phosphate was determined by the method of Kuttner and Cohen.[5] It is well known that the amount of inorganic blood phosphate changes rapidly in vitro (Kay and Byrom).[4] All analyses were made immediately after the withdrawal of the blood.

THE AVERAGE NORMAL CURVE

In chart 1 is shown the usual change in blood phosphate and sugar after the administration of 100 grams of glucose to a normal adult. The greatest depression of the phosphate occurred at the end of two hours. In the normal, the lowest point in the phosphate curve is always after the high point in the sugar curve. The decrease in blood phosphate in a normal individual after the

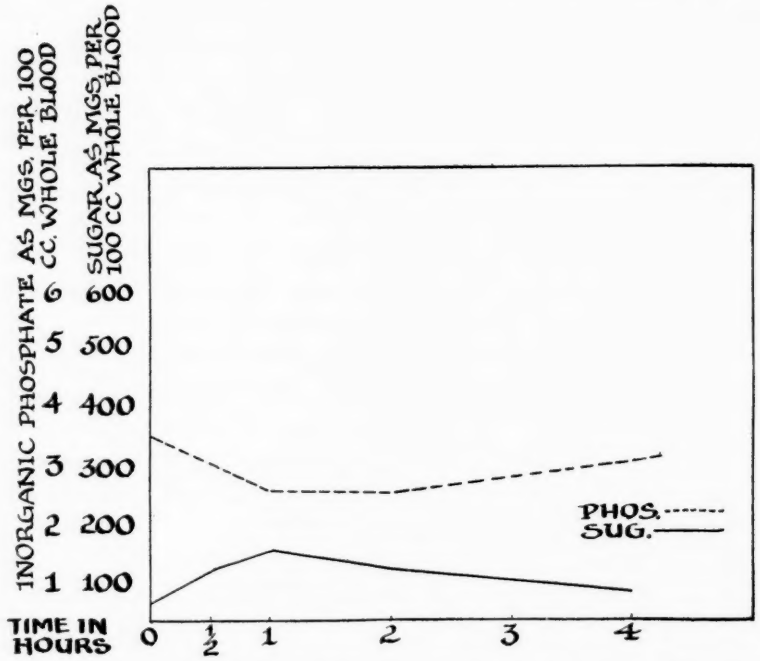

CHART 1. SUGAR AND PHOSPHATE CURVE IN AN AVERAGE NORMAL (PHOSPHATE CURVE FALLS AND RISES AGAIN)

administration of glucose averages about 0.7 mgm. per 100 cc. of whole blood, is seldom less than 0.2 mgm. per 100 cc. of whole blood, and seldom greater than 1.2 mgm. per 100 cc. of whole blood. The phosphate usually returns to nearly the original fasting level between three and four hours after the administration of the glucose.

In table 1 is shown an analysis of blood sugar and inorganic

blood phosphate curves in a series of forty-three patients in whom no known metabolic disorder was present. In the majority of these cases the sugar curves and inorganic phosphate curves

TABLE 1

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN FORTY-THREE PATIENTS HAVING NO KNOWN METABOLIC DISORDER

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves	4	2	2
Normal sugar curves	39	32	7
Total	43	34	9

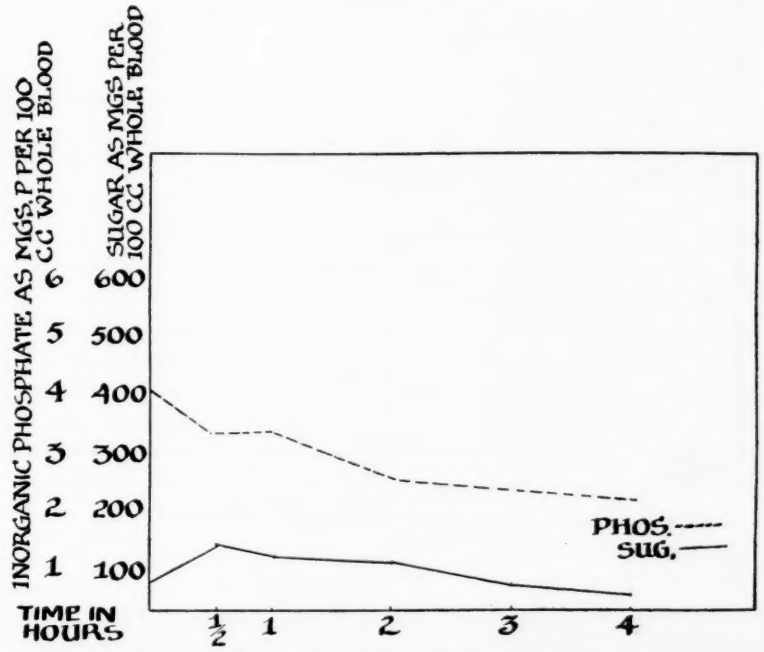

CHART 2. "DIABETIC" PHOSPHATE IN A NORMAL INDIVIDUAL (PHOSPHATE CURVE FALLS FOR FOUR HOURS)

did not deviate to any great extent from the average curve for normal individuals. Only nine of this group of forty-three patients showed definite changes in the type of phosphate curve.

An example of such deviation from the average in a normal individual is shown in chart 2.

In a large number of diseases the phosphate curve is frequently not normal. It has been found that abnormalities of the phosphate curve are confined chiefly to diseases of metabolism, such as diabetes, hyperthyroidism, hypothyroidism, pituitary disorders, and so forth.

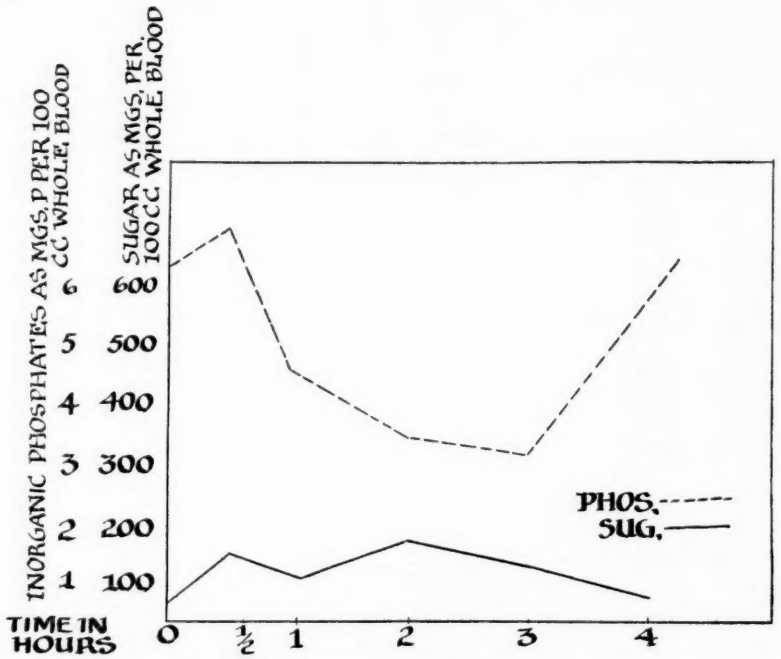

CHART 3. INORGANIC PHOSPHATE IN TETANY

TETANY

Our study of phosphate metabolism in post-operative parathyroid tetany and of the therapeutic value of the findings has been reported elsewhere.[6] In parathyroid tetany the phosphate is high and the drop is likely to be more marked than in normal individuals. When the phosphate depression is greatest, marked relief of symptoms occurs with decreased excitability as measured by electrical stimulation. The symptoms return when the phos-

phate returns to normal. This marked change is indicated in chart 3 which shows that the phosphate dropped from 6.9 to 3.4 mgm. per 100 cc. whole blood. The phosphate increased greatly again within the four-hour period. These changes are typical of this particular condition.

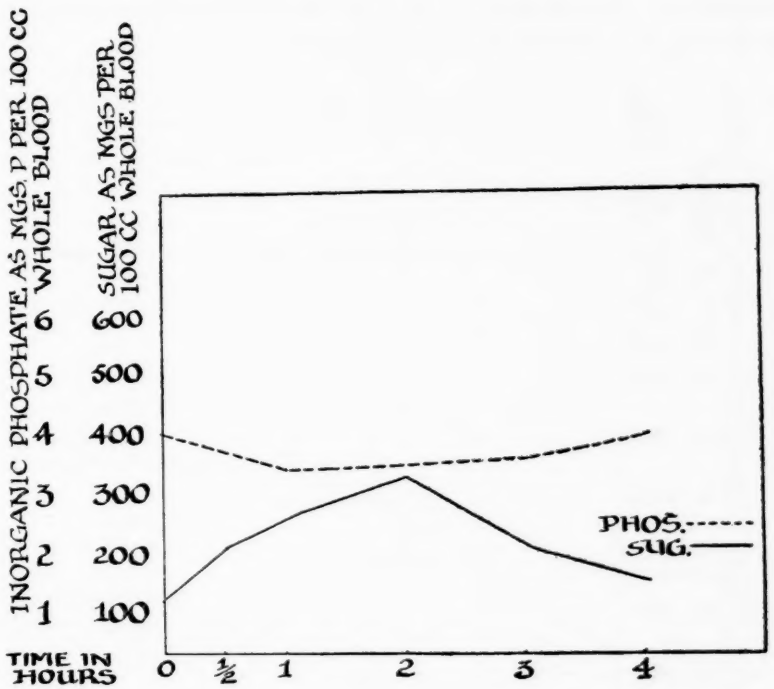

CHART 4. "NORMAL" PHOSPHATE IN A DIABETIC (PHOSPHATE CURVE FALLS AND RISES AGAIN)

DIABETES

The most marked changes in carbohydrate metabolism occur in diabetes. In a study of thirty-two patients, only 44 per cent (14 cases) or approximately one-half showed changes in phosphate curves which we could be justified in calling definitely abnormal. In 56 per cent (18 cases) the curves might well have been obtained from normal individuals. Each of these thirty-two patients was clinically definitely diabetic with a per-

sistently high sugar curve. An example of a so-called normal phosphate curve in a definitely diabetic patient is shown in chart 4. The depression of phosphate occurs at approximately the usual time and to approximately the usual extent. The return to the fasting level is also normal. The type of curve which Hartmann and Bolliger considered to be mildly diabetic is shown in chart 5. The phosphates are depressed but do not return to the fasting level before the end of the test.

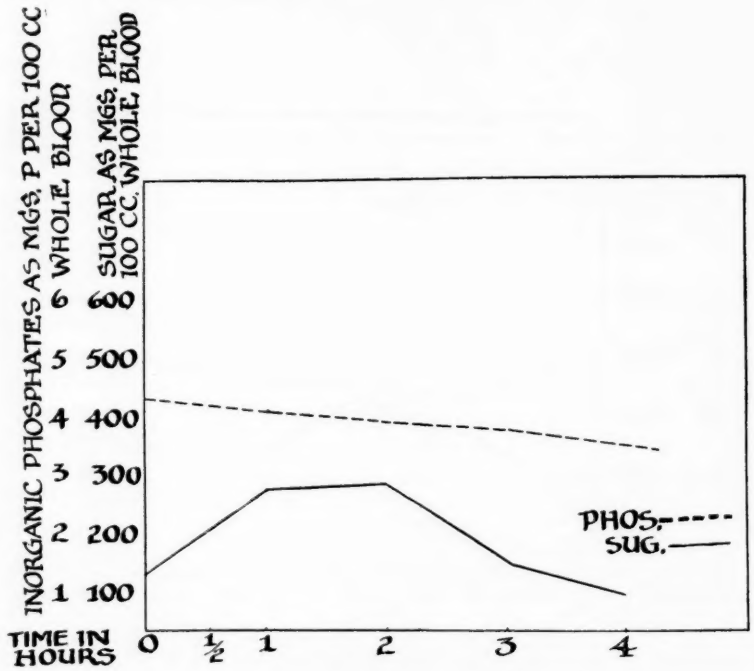

CHART 5. DIABETIC PHOSPHATE IN A DIABETIC (PHOSPHATE CONTINUES TO FALL FOR FOUR HOURS)

THYROID AND PITUITARY DISORDERS

The sugar and phosphate curves were also studied in forty-nine patients suffering from numerous metabolic disorders other than diabetes, such as hyperthyroidism, hypothyroidism, and pituitary dysfunction. In table 2 is shown an analysis of this group of curves. In thirty-two patients the sugar curves were normal, but 34 per cent of those patients showed abnormalities in phos-

phate metabolism. This percentage is definitely higher than that found in the group of patients in whom no diseases of metabolism were present. The sugar curve was high in nine cases but was not of the diabetic type. In this group of non-diabetics showing high sugar curves 33 per cent of the patients presented abnormal phosphate curves; that is a somewhat lower percentage of abnormal phosphate curves than was found in the diabetic

TABLE 2

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN FORTY-NINE PATIENTS SUFFERING FROM ENDOCRINE DISORDERS OTHER THAN DIABETES

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves:			
Hyperthyroidism	1	1	0
Hypothyroidism	3	3	0
Pituitary dysfunction	4	3	1
Total	8	7	1
Normal sugar curves:			
Hyperthyroidism	8	4	4
Hypothyroidism	11	9	2
Pituitary dysfunction	13	8	5
Total	32	21	11
High sugar curves:			
Hyperthyroidism	6	4	2
Hypothyroidism	1	0	1
Pituitary dysfunction	2	2	0
Total	9	6	3
Total	49	34	15

group. The shape of the abnormal phosphate curves of diabetics did not appear to be definitely different from those of the non-diabetics. Eight of the patients in the latter group presented low sugar curves, seven of whom showed normal phosphate curves.

ARTHRITIS

In forty-six cases of arthritis, table 3, sixteen patients presented definitely low sugar curves. We believe that this is not a coincidence, but that arthritics have a greater tendency toward dis-

turbed carbohydrate metabolism than most groups of patients. Seven of the sixteen arthritics with low sugar curves showed also abnormalities in phosphate metabolism. In 70 per cent of the forty-six cases the phosphate curves were normal.

TABLE 3

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN FORTY-SIX CASES OF ARTHRITIS

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar	13	6	7
Normal sugar	27	22	5
High sugar	6	4	2
Total	46	32	14

TABLE 4

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN PATIENTS SUFFERING FROM MORE THAN ONE DISORDER

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Arthritis and diabetes:			
High sugar curves	4	3	1
Thyroid dysfunction and arthritis:			
Low sugar curves	1	0	1
Normal sugar curves	6	5	1
High sugar curves	2	1	1
Total	9		
Thyroid dysfunction and diabetes:			
High sugar curves	13	5	8
Thyroid and pituitary dysfunctions:			
Low sugar curves	2	2	0
Normal sugar curves	3	2	1
High sugar curves	1	0	1
Total	6		

PATIENTS SUFFERING FROM SEVERAL METABOLIC DISORDERS

There were thirty-two patients in the total series of 230 who suffered from more than one disorder which we thought might affect their metabolism, namely; arthritis and diabetes, thyroid

dysfunction and arthritis, thyroid dysfunction and diabetes, and pituitary and thyroid disorders (see table 4).

In the group of patients in whom diabetes was associated with thyroid dysfunction 63 per cent of the phosphate curves were normal, which is a slightly greater percentage than that found in the diabetic patients. The shape of the phosphate curve was again not at all unique.

These patients suffering from arthritis associated with diabetes presented usual diabetic sugar curves.

In the group of patients in whom thyroid dysfunction was associated with arthritis and in the group in which both thyroid and pituitary disorders were present the number of abnormal

TABLE 5

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN ELEVEN PATIENTS AFFECTED WITH MISCELLANEOUS ENDOCRINE DYSFUNCTIONS

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves	4	4	0
Normal sugar curves	6	3	3
High sugar curves	1	1	0
Total	11	8	3

phosphate curves was unusually large. The shape of the phosphate curve, however, did not differentiate this group from any other group of abnormal phosphate curves.

OTHER ENDOCRINE DISORDERS

We also studied a group of eleven patients suffering from various metabolic disorders including hyperadrenalinism, ovarian dysfunction and hypometabolism. Eight patients in this series presented normal phosphate curves. The sugar curves varied as follows: four patients presented low sugar curves, six showed normal sugar curves and one a high sugar curve, which was not of the diabetic type (see table 5).

DISCUSSION

We had hoped that the phosphate curves might be of more definite value in the differential diagnosis of various metabolic

disorders. A consideration of various metabolic processes in which phosphates play some rôle leads us to believe, however, that the results of this investigation are not at all surprising. The changes in blood phosphate observed after feeding sugar are due chiefly to the part played by phosphorus in carbohydrate anabolism. Carbohydrate anabolism is very definitely disturbed in diabetes, hence we would expect frequent changes in phosphate metabolism. Phosphates, however, play a large part in the metabolism of fats, nucleoproteins, and in the metabolism of muscle. They also act as buffers in the tissue and tissue fluids. Since all of these metabolic processes are variable, it is not surprising to find considerable irregularity in the changes of the phosphates in the blood after the administration of glucose. It may be that the response would have been somewhat more regular if the glucose had been administered intravenously as in the cases studied by Hartmann and Bolliger. It seems probable, however, that in their relatively small group of patients Hartmann and Bolliger happened to get more constant results and results which are somewhat atypical. We feel that it is quite impossible to place much value on the phosphate curve in the differential diagnosis of metabolic disorders.

SUMMARY

1. The phosphate changes in the blood of normal individuals after the administration of glucose show considerable regularity.

2. The changes in blood phosphate after the administration of glucose to patients suffering from metabolic disorders frequently differ from the changes in blood phosphate in normal individuals.

3. It is impossible to make a definite differential diagnosis in various metabolic disorders by means of the phosphate curve.

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CLINICAL EVALUATION OF BLOOD PHOSPHATE AND SUGAR TOLERANCE CURVES

An Analysis of 500 Clinical Cases

F. W. HARTMAN and D. P. FOSTER

From the Departments of Laboratories and Medicine, Henry Ford Hospital, Detroit, Michigan

In previous publications[2,3] the experimental basis for the use of the inorganic blood phosphate curve during the sugar tolerance test was presented together with the analysis of sixty curves from different cases. This study records similar observations and the analysis of curves from 500 clinical cases of the incipient or prediabetic group. These patients had family histories of diabetes, or asthenia and poor endurance, or were overweight or underweight. Some gave a history of boils, carbuncles, paresthesias and pruritis.

With sixty curves illustrating the various types of abnormal carbohydrate metabolism already reported, it seemed that the most exacting test to which the sugar tolerance curve and the inorganic phosphate curve could be subjected would be the application to the borderline or potential diabetic group. The importance of correct diagnosis at this stage has been emphasized by Johns,[4] Sherrill[5] and others because, with early recognition of abnormal carbohydrate metabolism, severe diabetes may often be avoided through simple dietary and hygienic management. The following procedure has been applied in more than 500 instances on patients sent to the metabolic division for study.

TECHNIC

The individuals were in the post-absorbtive period and 35 grams of glucose in 50 per cent solution were injected intravenously in five to six minutes. Blood specimens were withdrawn just before the injection and fifteen, forty-five, seventy-five, and one-hundred fifty minutes after the injection. The blood sugar values were determined by the method of Folin and Wu and the blood phosphates by the method of Benedict and Theis.

In this series, more than half showed little or no abnormality in either the glucose or phosphate curves. Analysis of their histories, physical examinations and follow-ups gave no evidence of abnormal carbohydrate metabolism. Four curves from this group have been selected as typical and fifty curves have been averaged to produce a composite normal. (Fig. 1 and fig. 5, curve 1.)

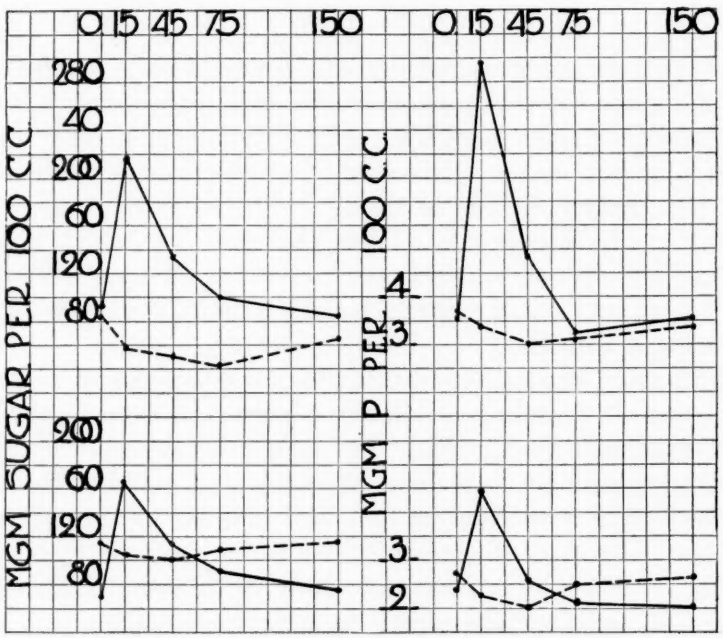

FIG. 1. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING NORMAL CARBOHYDRATE METABOLISM

In this and all other curves, time in minutes is indicated at the top of the figure.

One hundred of these patients were overweight from twenty-five to 100 pounds. Figure 2 shows four curves which typify the group and the composite curve (Fig. 5, curve 2) representing 100 patients. If the glucose curves are contrasted with those in fig. 1 it is seen that the blood sugar rises from 20 to 100 mgm.

higher in fifteen minutes, despite the obesity in this group, and the fall is much more gradual giving a large obtuse angle. The fasting level is reached in all at the end of two and one-half hours. The curve of inorganic blood phosphates in this group shows an average drop of 0.5 mgm. with the lowest point reached

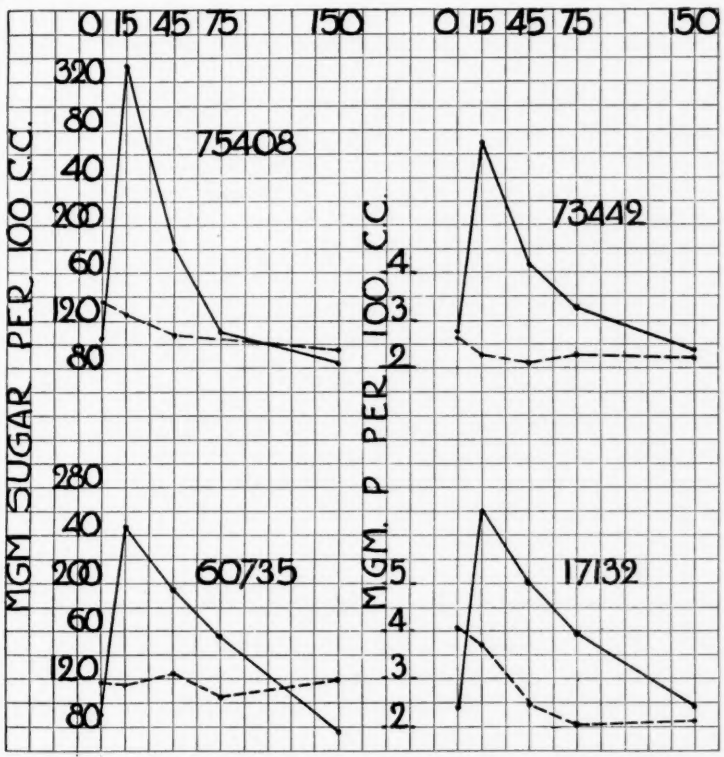

FIG. 2. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING VARIATIONS FROM THE NORMAL IN OBESE PATIENTS

in forty-five minutes, but there is slight tendency to recovery. In the light of experimental data the glucose curve could be explained on the basis of hepatic insufficiency but the phosphate curve indicates diminished available insulin. The clinical résumés for typical cases in this group, four of which correspond with the curves, follow:

Case 88287. Female; aged fifty-nine. Forty-three pounds overweight. Complaint: Eye trouble (cataracts). Past history: Polydipsia, polyuria, paresthesia and cramps in legs. Under dietary regulation the fasting blood sugar decreased from 119 to 94 mgm. per 100 cc. and her general condition improved.

Case 17132. Male; aged forty-eight. Twenty-nine pounds overweight. Complaint: Fatigability. Family history: Father died of diabetes. Past history: Weakness, polydipsia, in 1922 fasting blood sugar 78 mgm. per 100 cc. No glycosuria at that time. In 1928 symptoms were increased and glycosuria ++++.

Case 73442. Male; aged thirty-eight. Overweight sixty-nine pounds. Complaint: Tired feeling. Past history: Weakness and nervousness. Low caloric and low carbohydrate diet relieved symptoms.

Case 60735. Male; aged thirty-five. Overweight twenty pounds. Complaint: Recurrent conjunctivities. Past history. Fatigability, hypotension. Improvement on low caloric, low carbohydrate diet.

Case 75408. Female, aged fifty-eight. Thirty-three pounds overweight. Complaint: Pruritis vulvae. Past history. Pruritis, polyphagia, polydiasia and paresthesia. A fasting blood sugar of 118 mgm. per 100 cc. in August, 1926 was reduced to 91 mgm. per 100 cc. by March, 1928. Weight was reduced eighteen pounds by dietary regulation.

Case 94592. Female; aged sixteen. Overweight fifty pounds. Complaint: Marked weakness. Past history: Gained sixty-two pounds in ten months after menstruation began. Polyphagia, polydipsia, and dry mouth. Regulation of diet produced loss of eleven pounds in weight and brought the blood sugar down to 71 mgm. per 100 cc.

One hundred two of the patients examined were of normal weight or undernourished. Figure 3 gives curves from four average cases in this group. (see also fig. 5, curve 3). The rise of blood sugar at the end of fifteen minutes is from 88 to 238 mgm. or 43 mgm. more than the increase in the normal group (fig. 1) and 18 mgm. more than the increase in the overweight group (fig. 2). The fall of blood sugar is more gradual than the normal, leaving an obtuse angle between the two sides of the curve. This angle is more acute than in the overweight group. The phosphate curve shows only 0.25 mgm. depression, the low point is reached after one hour and fifteen minutes and there is little tendency toward recovery. Here the relatively larger amount of glucose injected apparently accounts for the greater increase in the fifteen-minute blood sugar. Probably the increased storage

accounts for the more acute fall and return to the fasting level in two and one-half hours. The phosphate curve again indicates available insulin in limited amounts. The clinical outlines for this group follow:

Case 71721. Male; aged thirty-nine. Complaint: Weakness and nervousness, polyphagia +++, polydipsia ++, polyuria ++, glycosuria ++++, asthenia +++, January, 1926 glycosuria ++++, fasting blood sugar 101

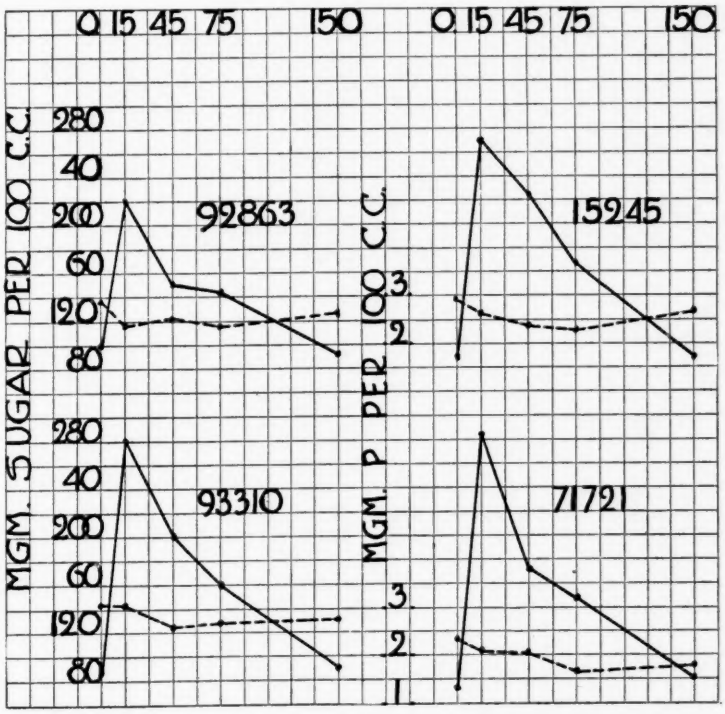

FIG. 3. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING VARIATIONS FROM THE NORMAL IN PATIENTS OF SUBNORMAL OR NORMAL WEIGHT

mgm. per 100 cc. July, 1927, after dietary regulation, fasting blood sugar 81 mgm. per 100 cc., urinary sugar negative. Felt much improved.

Case 15245. Male, aged sixty. Family history: Family all overweight, father obese, grandfather died of diabetes, sister has diabetes. Past history: Polyphagia +++, polydipsia +++, polyuria ++: Present examination: Arteriosclerosis, hypertension and marked paresthesia of extremities.

Case 93310. Male; aged thirty-two. Complaint: Weakness and vague, shifting pains. Past history: Polydipsia, polyphagia, polyuria. Twenty pounds underweight. Course: Improvement in symptoms with low carbohydrate diet. Blood sugar continued normal.

Case 47574. Female, aged forty. Complaint: Glycosuria. No symptoms suggestive of diabetes but fasting blood sugar at times reached 111 mgm. per 100 cc. Urine and blood returned to normal after dietary regulation.

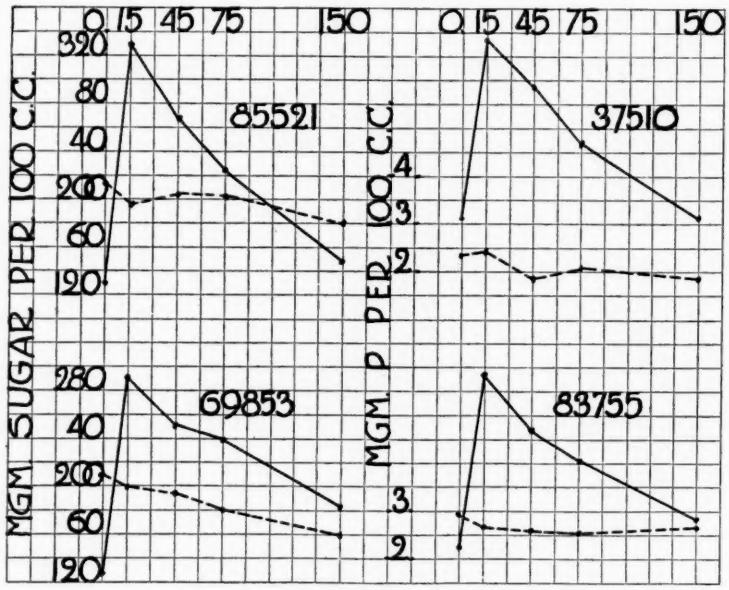

FIG. 4. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING VARIATIONS FROM THE NORMAL IN PATIENTS WITH DIABETES

Case 54789. Male; aged fifty-five. Complaint: Impotence. Past history: Weakness and paresthesia. Present examination: Arteriosclerosis and myocarditis. Fasting blood sugar 100 mgm. per 100 cc.

Case 92863. Male; age sixty-three. Complaint: Glycosuria ten years duration. Family history: Mother obese, father died at age of 56 of diabetes. Present examination: Arteriosclerosis, hypertension, glycosuria +++.

Although sugar tolerance tests are not indicated in diabetes, twenty-five mild diabetics were included in this study and are represented in figure 4 and figure 5, curve 4. The individual charts show the expected sharp rises in the blood sugar in the first

fifteen minutes with formation of a very obtuse angle between the two sides of the curves and tendency toward plateau formation in the descending portion of the curve. In some instances the two and one-half hour reading does not reach the fasting level. The phosphate curves show a tendency to straighten out with continued depression at the end of the observation period, indi-

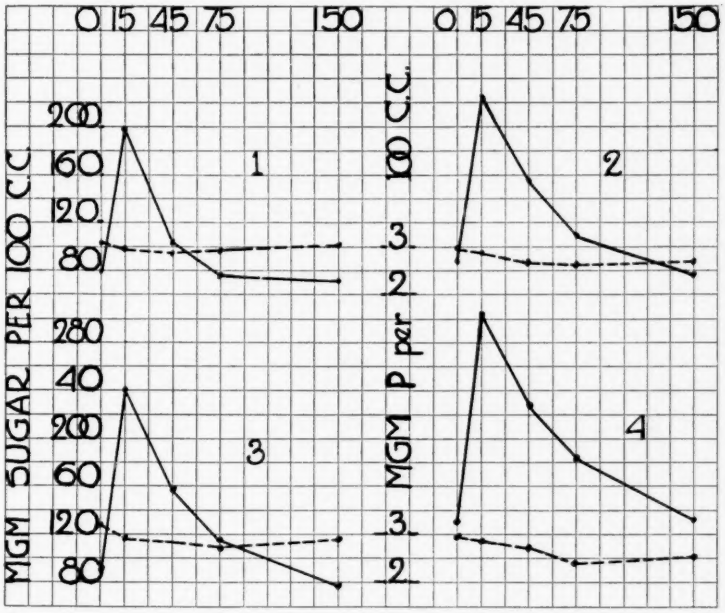

FIG. 5. COMPOSITE CURVES SHOWING GLUCOSE AND PHOSPHATE CURVES UNDER VARIOUS CONDITIONS

Curve 1, normal patients; curve 2, obese patients; curve 3, abnormal patients of subnormal or normal weight; curve 4, patients with diabetes.

cating less available insulin than in any of the groups considered. Nos. 83755 and 37510 show all the phosphate values charted on almost a straight line suggesting strongly that the diabetes was severe since similar curves were obtained on depancreatized dogs. The clinical outlines of this group follows:

Case 83755. Female; aged fifty-four. Complaint: Diabetes mellitus. Family history: No history of diabetes. Past history: Polyphagia, polydipsia,

polyuria, nocturia. Asthenia and poor endurance, dry mouth, paresthesia, pruritis. Present examination: Obese (one hundred sixty-five pounds, 20 per cent overweight). Fasting blood sugar reduced from 151 mgm. per 100 cc. to 90 mgm. per 100 cc. on diet of 2000 calories.

Case 69853. Female; aged sixty-three. Complaint: Eczema for six years. Family history: Family overweight. Father died of heart disease. Past history: Polyphagia, polydipsia. Weight 241; estimated weight 146. Boils during childhood.

Case 70434. Aged fifty-one; weight 181 pounds; estimated weight 141 pounds; blood sugar 200 mgm. per 100 cc. Complaint: Pain in stomach. Asthenia, poor endurance, occasional pruritis, "neuritis-like pains," oral sepsis, severe grade. Family history: Family all obese. No urinary sugar before tests were done. Diet: Protein, 60 grams; fat, 80 grams, carbohydrates, 100 grams, which was tolerated. Blood sugar, 83 mgm. per 100 cc., one month after treatment was started. Later dietary indiscretion caused a blood sugar of 190 mgm. per 100 cc. Weight on 5/17/27, 147 pounds; blood sugar 99 mgm. per 100 cc.

Case 37510. Age fifty-eight. Weight, 148 pounds; estimated weight, 153 pounds. Glycosuria in 1923. Classical diabetic history with all the "polys" and leg cramps, oral sepsis, chronic eczema. Diet: Protein, 50 grams; fat, 140 grams; carbohydrate, 50 grams. Blood sugar ranged between 105 mgm. and 157 mgm. per 100 cc.

Case 85521. Aged fifty-one. Complaint: "gas in abdomen." Family history: Mother obese. Past history: Hearty appetite, dry mouth numbness in hands. On restricted diet blood sugar fell from 133 mgm. per 100 cc. to 87 mgm. per 100 cc. after fasting.

Case 74516. Female, aged thirty-one. Always obese. One hundred seventy pounds at sixteen years of age; 255 pounds now; estimated weight 145 pounds. Family history: Father's weight 190 pounds; brothers' weight 190 pounds. Past history: Polydipsia at times. 3/11/26—Blood sugar after fasting 121 mgm. per 100 cc. 5/11/26–Blood sugar after fasting 99 mgm. per 100 cc. Weight now, 239 on 1000 calorie diet.

SUMMARY

(1) Five hundred combined glucose tolerance and phosphate curves were taken on patients considered potential diabetics. One hundred were from individuals twenty-five to one-hundred pounds overweight. These showed an increased rise of the blood glucose with slow fall while the inorganic phosphates decreased moderately with slow recovery.

(2) One hundred two combined glucose tolerance and phosphate curves from individuals, normal in weight or under-

nourished, showed high elevation of the glucose curve with gradual return to the fasting level while the phosphate curve showed only slight depression with slight recovery.

(3) Twenty-five combined glucose tolerance and phosphate curves on mild or moderate diabetics showed typical diminished glucose tolerance curves. The phosphate curves showed slight and continued depression.

(4) The curve of inorganic phosphates is a valuable supplement to the glucose tolerance curve in the diagnosis of abnormal carbohydrate metabolism.

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THE PATHOGENESIS OF TUBERCULOUS HEMOPTYSIS*

A Clinical-Pathological Investigation

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Hemorrhage from the lungs is the most appalling manifestation of pulmonary tuberculosis. The majority of the victims of this wide-spread disease experience this frightful symptom at least once and often repeatedly, and death results immediately in thousands of cases each year. Clinical observations of this phenomenon, consequently, have been made in abundance from the earliest times,[11] and a mere bibliographic list of the published contributions constitutes a good sized book. In spite of this wealth of recorded data and elaborate discussions, the anatomical location, the pathological processes and the physiological mechanisms that are concerned in tuberculous hemoptysis still require further elucidation.

Hemorrhage from the lungs may conceivably arise from either the bronchial or the pulmonary circulation, and may be arterial, venous, or capillary in origin. In patients who come to autopsy within a short time after a hemorrhage from tuberculosis, however, the bleeding point is uniformly located in the pulmonary circulation and practically never seen in one of the bronchial vessels, except in the rare cases of ulceration of a calcified hilum gland through the trachea or primary bronchi.[5] This is the reverse of the findings in hemorrhage from general hypertension or ulcerative bronchitis. Moreover, the bleeding vessel is found to be a branch of the pulmonary artery and not a vein or capillary, as is apt to give rise to hemorrhages in patients with mitral stenosis or pneumonia.

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In the absence of necropsy observations, it cannot be proven that this also holds true in the slight streaking and initial hemorrhages in less advanced instances of tuberculosis, plausible though this may be. Frank hemoptysis in the course of the disease, however, can be shown by roentgenogram to arise mainly from chronic fibroid phthisis with cavitation. When such patients come to autopsy, the lesions are readily demonstrated and the bleeding, when recent, is found to arise from one of the cavities.[20] This was confirmed in more than two score successive autopsies performed at the Olive View Sanatorium on patients who had had a history of hemoptysis while in the institution. Profuse tuberculous hemorrhage, particularly when fatal, always proceeds from a branch of the pulmonary artery. This also was corroborated in six fatal instances of hemoptysis at the Olive View Sanatorium.

It is often stated that the cause of pulmonary hemorrhage in tuberculosis is the erosion or ulceration of the blood vessel walls by a developing tuberculous process.[6] Since the tubercle bacillus and its products are capable of producing death with subsequent softening and liquefaction of tissues as resistant even as bone, it is assumed that the same process, acting on a blood vessel wall, may produce similar softening and consequent rupture of the vessel through which the blood can rush out. Hemorrhage, then, would be a direct result of caseation affecting the blood vessel.

If this were true, the forms of tuberculosis characterized by caseation, the exudative, rapidly progressive types in which large portions of the lungs may undergo massive coagulation necrosis in a short time, would be the most frequently affected by pulmonary hemorrhage. Experience, however, teaches that this is, in fact, the rarest of occurrences. In miliary tuberculosis, in the galloping consumption of primitive peoples coming into contact with the disease for the first time, and in the childhood type of tuberculosis in general, the absence of pulmonary hemorrhage is so striking as to be repeatedly remarked.[15] Rapid spread may, and often does, follow the hemorrhage, but hemorrhage in the course of a tuberculous process in which no resistance can be

demonstrated is indeed rare. On the contrary, it has been repeatedly noted that hemorrhage is most apt to affect the fibroid types of the disease, the proliferative forms in which the advance of the lesions are slow, and the caseation necrosis scant. In fact, it is not uncommon for apparently completely arrested cases to suffer from sudden hemorrhages.

Cloudy swelling, necrosis and liquefaction of tissues results in response to many different kinds of infection, rarely, however, is it accompanied by profuse open bleeding. The vessels in these instances are usually sealed by intravascular thrombosis and organization, with obliteration of the lumen, long before the vessel walls give way. In extrapulmonary tuberculosis the same process occurs. And more than a century ago it was pointed out that in pulmonary tuberculosis the blood vessels bordering on or traversing tuberculous cavities in the lungs are obliterated by thrombosis and endarteritis in the lumen, as well as by the compression of the contracting fibrous tissue around the adventitia. The exceptions to this observation are mainly vessels not affected by the tuberculous process, but enveloped by the resultant fibrous contraction or developed in newly forming granulation tissue.

The naïve explanation that the tuberculous process simply erodes through previously intact blood vessels, and thus produces hemorrhage is, therefore, in all probability incorrect. This applies whether the vascular injury is ascribed to ulceration and caseation due to the tubercle bacillus, its toxic products, other organisms and their toxins,[21] or the ferments and cytolytic enzymes set free from the cells already destroyed.

The idea that the blood in pulmonary hemorrhage may arise by simple diapedesis or sanguinous exudation from actively or passively dilated alveolar or bronchial capillaries as a result of vasomotor,[8] nervous or endocrine derangements in the course of clinical tuberculosis, or from toxic or mechanical factors due to the disease process itself has been advanced by a host of writers,[16] but little evidence has been submitted to support these conceptions. Changes in the composition and behavior of the blood itself have also been invoked to explain tuberculous hemoptysis and means of therapy based on these ideas have been vigorously

championed and are still widely used.[14] Most workers have failed to confirm these claims; the clotting time and bleeding time in patients with advanced tuberculosis is more often diminished, rather than increased, and the blood clot shows normal retractility.

Pulmonary hemorrhage, when investigated on the postmortem table, is found to proceed from the wall of an old cavity in the affected lung, usually a thick, firm, fibrous wall, showing little evidence of active disease, but well organized granulation tissue with marked evidence of contracting scar and tension effects resulting from it. Demonstrable lesions in the pulmonary blood vessels at the site of the hemorrhage have been reported by many workers during the past century.[7, 9, 12] That most frequently noted is an aneurysm developing on a branch of the pulmonary artery lying in the wall of the cavity.[19] Patent blood vessels in such cavity walls are often thick walled beyond the normal, and bound down by fibrous bands which may in places produce partial or complete occlusion of the lumen. The resulting interference with the nutrition rather than any local infection of the vascular tissue itself may cause the weakening of the pulmonary arterial wall and the formation of an aneurysm. Occasionally this aneurysm may become lined with a laminated clot undergoing organization, similar to that found in healing aortic aneurysms. Such lesions are usually found on the larger branches of the pulmonary artery, but may develop in the smaller ones and even in the capillaries.[17]

The idea that these aneurysms develop from weakened areas on the vessel walls when the tuberculous process or other infective lesion is advancing too rapidly for the normal endarteritis and thrombosis to obliterate the lumen of the vessel, is untenable in view of the rapidity with which this latter process does occur in cases of really rapidly advancing phthisis. The rather fantastic suggestion that the increase in fibrinogen actually present in most cases of tuberculosis forms a false coating on the inner wall of the vessel, and thus interferes with its nutrition, resulting in weakening of the vessel wall and the formation of the aneurysm is interesting but improbable.[13] The loss of support to the

vessel wall from the excavation of pulmonary tissue which had previously occupied the cavity space is inconsiderable in view of the elasticity and ready collapsibility of the lung.

The weakening of the blood vessel wall in chronic pulmonary tuberculosis by the contracting scar interfering with the nutrition of the blood vessel itself gives only a part of the picture necessary for the production of a pulmonary hemorrhage. The other factor involved is the blood pressure existing within the vessel. It is an old observation that cerebral hemorrhage is almost confined to patients with increased intraarterial tension. It would not be surprizing, therefore, to learn that pulmonary hemorrhage occurs particularly in the presence of increased intrapulmonary arterial pressure.

Under normal conditions the pressure existing within the pulmonary vessels is low, in fact much less than half of the tension of the systemic circuit. In chronic pulmonary tuberculosis, however, much of the vascular bed through which the blood must pass in going from the right ventricle to the left auricle is closed by intravascular thrombosis and compression, as well as by actual destruction of large parts of the lung fields. Since all of the blood of the body must, nevertheless, pass through this pathway to reenter the heart, the velocity in the remaining vessels still open must be increased, and accordingly the pressure in the pulmonary artery is probably increased.

The amount of blood lost in tuberculous pulmonary hemorrhage is less than one ounce in about half of the instances reported, and rarely more than ten ounces. With repeated hemoptyses the blood loss to a patient may become considerably larger, but still is seldom sufficient to account for death from exsanguination. The immediate danger from pulmonary hemorrhage is that of suffocation from the large amount of blood coagulating and obstructing the bronchi and trachea. Instances of the removal of such blood casts of the trachea and bronchial tree have been reported, with subsequent recovery of the patient.[22]

Less dramatic but more important for the future course of the disease in most cases, however, is the aspiration of bloody material to distant parts of the lung. It has been shown that

the aspiration of sterile blood into normal lung tissues may set up considerable reaction, but this will eventually subside. When the blood is mixed with tuberculous material from the cavity contents, however, bronchiogenic spread of the disease process, sometimes pneumonic in type, is quite likely to occur.[2] Another accompaniment of pulmonary hemorrhage that is occasionally encountered consists of embolic phenomena, cerebral accidents, purpura, and so forth following the hemorrhage. This perhaps, suggests the formation of venous thrombi subsequent to infarction from thrombosis in the bleeding artery, rather than actual rupture or coagulation in a diseased pulmonary vein.

More than 4,000 instances of pulmonary hemorrhage have been recorded at the Olive View Sanatorium during the past four years, arising among 450 of the nearly 3,000 patients who have been cared for in the institution during this time. The marked periodicity in their occurrence, 80 per cent of them being reported on less than one-third of the days included in this study, suggested the possible importance of external factors in their precipitation, and the data were accordingly investigated from this point of view.[3]

The hourly variation in the incidence of pulmonary hemorrhage as revealed in this study was unexpected but unmistakable and consistent. The general impression that pulmonary hemorrhages are likely to occur during the night seems to be based more on the amount of disturbance they cause than upon their actual frequency. A confirmatory study made at the Duarte Sanatorium and analyses of other data agreed perfectly with these findings. During the walking hour, from 6:00 to 7:00 a.m., there were nearly three times as many hemorrhages recorded as during any of the hours during the night, or during the afternoon rest period.

The coughing that ensues when the patients arise from their sleep and try to expel the secretions which accumulate during the night not only involves a considerable amount of movement of the lungs and other structures, with sudden marked changes in intrapulmonary strains and stresses, but also increases the blood pressure both in the systemic and in the pulmonary circulation.

The increased activity at mealtime rather than any physiological effect of the ingestion of food may account for the increased numbers of hemoptyses seen at these hours. The lowest numbers of hemorrhages appear during the periods, both day and night, when the patients may be expected to be most completely at rest. The varying curve of vital functions during the day, the relationships of the curve of temperature, pulse and blood pressure are in accord with such a view.

The entire series showed no particular predilection for any time of the year, in marked contrast to previous observations.[1] This may be accounted for by the peculiarities of the California weather, the absence of real cold weather, freezing never having occurred at this sanatorium during the entire period, and by the consequent constancy of the environment, clothing, diet, and so forth. The variation on different days of the week is very slight; the increases on the days preceding visiting days are too small to be significant.

The incidence of pulmonary hemorrhage appears to be greatest on the days of the highest barometric pressure, and lowest on the days when the barometer is low. The uniformity of this relationship, and the absence of any significance in the mere amount of fluctuation of the barometer, either rise or fall, is in sharp contrast to the data previously reported by other workers,[4,10] but is here too marked and consistent to be disregarded. It is recalled that the systemic blood pressure increases with increase in the barometric pressure,[18] and vice versa, but that this also occurs in the pulmonary system, to any appreciable extent, although plausible, is still unproven.

Tuberculous hemoptyses also occured in greater numbers on the days of the highest maximal temperature. The relationship of the temperature to the symptoms of tuberculosis has been often discussed but is still obscure. It may be recalled that the incidence of hemoptysis need not coincide with that of the other symptoms. As noted above, few uncomfortably cold days occurred during this series.

The humidity bears no constant relationship to the incidence of pulmonary hemorrhage in this study. Since this is a dry

climate, the effects of excessive moisture may not be discerned in the figures here available. There appears to be some decrease in hemorrhages, however, with increases in the velocity of the wind, the greatest number occurring on the days with little or no air movement.

Although pulmonary hemorrhage does not necessarily indicate activity in a tuberculous patient, and may even evidence resistance and fibrosis, nevertheless its occurrence is in itself a dangerous affair, and is responsible for about one-tenth of the deaths in this group, or about 3 per cent of all the deaths in the Sanatorium by immediate suffocation or exsanguination, while a somewhat greater number died within a week or so thereafter. Thus, in the last 150 autopsies performed at the Olive View Sanatorium, in forty-three there was a record of pulmonary hemorrhage during the stay of the patient, while in fifteen there was blood in the bronchial passages or in cavities, indicating that the patients had died in hemorrhage. Every one of these forty-three cases showed fibrous or fibro-ulcerative tuberculosis with cavitation. There was not one that did not show some pleural involvement, and the pleural space was practically obliterated in nearly half of them. In the remainder there were usually dense adhesions on the side of the bleeding. Pneumothorax on the affected side, therefore, would have been impossible in practically all of the hemorrhage cases that came to autopsy. On the other hand, the hemorrhage cases that did receive successful pneumothorax treatment while at Olive View showed a somewhat lower mortality rate than those not so treated.

During the past year the bleeding point was located in six instances of fatal hemoptysis. In each case it consisted of an opening, either a linear rupture or a pinhole perforation, in a dilatation or aneurysm of a branch of the pulmonary artery. In only one instance was it in the right lung, in the remaining five it was found in the left side. In the entire series of forty-three cases, the bleeding was just twice as commonly on the left side as on the right. In one instance two small aneurysms were found on the same vessel, about a centimeter apart. In another the aneurysm arose from the pulmonary artery a short distance

from the main stem. In no case was erosion or perforation found in a patent vein. In each case there had been marked contraction evident in the involved lung.

A modern conception of the pathogenesis of tuberculous hemoptysis recognizes that the tuberculous process may be quiescent or even completely healed at the site of the rupture in the vessel, and that the hemorrhage is due rather to secondary changes that have occurred, both in the artery affected and in the entire circulatory-respiratory mechanism, as a result both of the disease and of climatic and other factors independent of it, instead of to the active infection caused by the acid-fast agent itself.

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A TYNDALLMETER-COLORIMETER FOR BIOLOGICAL USE AND SOME APPLICATIONS TO TURBIDIMETRIC AND COLORIMETRIC MEASUREMENTS IN MEDICINE

I. Description of the Tyndallmeter-Colorimeter for Biological Use

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It is desirable that an instrument be available for turbidimetric and colorimetric determinations on small samples of biological suspensions and solutions. It should have the following characteristics:

(1) The most sensitive optical methods of measurement should be employed in the new instrument. The instrument should be designed to utilize the great sensitivity which may be obtained by the measurement of the scattered-reflected light produced by turbid suspensions and known as the Tyndall phenomenon. (This very sensitive means of measurement has recently fallen into oblivion due to the increasing use of colorimeters as turbidimeters.)

(2) The instrument should be capable of measuring the optical properties of solutions over a wide range of dilutions.

(3) Turbidimetric and colorimetric determinations should be made by direct photometry on the substances in test tubes without removing the plug protecting the contents of the test tube and without loss of any portion of the sample, thereby obviating the present day practice of changing the substance to another vessel and immersing a plunger therein.

(4) Test tubes of varying size should be used in the same instrument for these determinations.

(5) One source of radiation (an electric lamp) should be used for both the known and unknown substances to automatically compensate for changes in the energy and other factors influencing the intensity or quality of the source of light.

(6) The standard of reference to which the turbidity or color of the suspension or solution of unknown strength should be compared should be a physical unit which will not change with time nor deteriorate.

DESCRIPTION OF THE TYNDALLMETER-COLORIMETER

The "Tyndallmeter-Colorimeter for Biological Use" was primarily designed to make possible the use of laboratory and

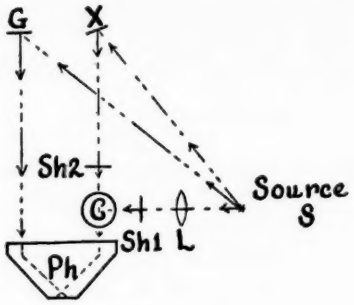

FIG. 1

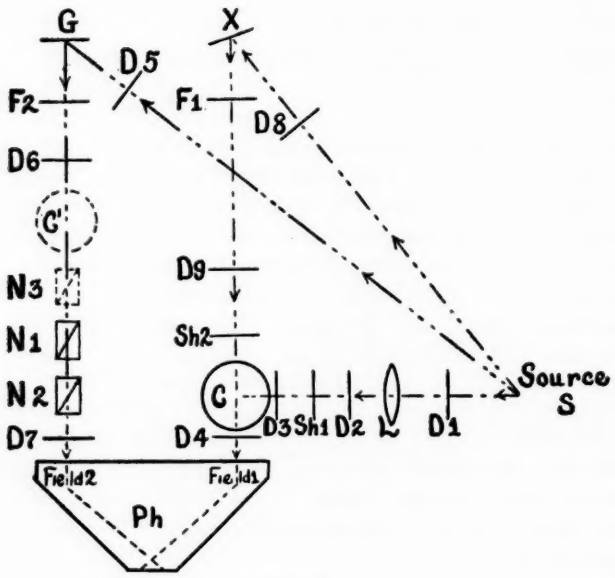

FIG. 2

bacteriological test tubes as containers for the fluids to be studied and to utilize certain optical principles in the manner described. The method of photometry is optional and many methods other than that described in this paper may be used depending on the

personal preference of the operator and the requirements of the laboratory.

The essential principles of the Tyndallmeter-Colorimeter are illustrated in figure 1. The method of photometry used in this work is illustrated in figure 2. In figure 2 are also included many refinements for certain specialized problems which are not required in many routine bacteriological and clinical laboratory determinations. In the following description of the instrument, various photometric devices which may be substituted for those used in the instrument described will be suggested.

I. The optical system (figure 2)

A. As a Tyndallmeter.

The source of light (S) is a concentrated filament electric lamp and sends out three beams of light. One beam of light (hereafter to be referred to as the incident beam of light) is made parallel by means of a short focal length lens (L) and passes through a small aperture* in a diaphragm (D3) in front of cell (C). The test tube containing the turbid suspension is placed in cell (C). The scattered-reflected light produced by the turbid suspension in the test tube is observed through another small aperture* in a diaphragm (D4) in front of the side of the cell (C) adjacent to the side of the first aperture. (The cell is described in detail in section III.) This observation may be made at any angle (usually 90 degrees) with the incident beam of light.

The second beam of light coming from the source (S) falls on a suitable diffusing surface plate (G). The scattered-reflected light produced by the turbid suspension in cell (C) and the light on plate (G) are now brought into precise juxtaxposition and photometered by means of the photometer cube and a system of Nicol prisms (to be described in detail in section II).

D1, D2, D3, D4, D5, D6 and D7 are diaphragms with apertures for adjusting the illuminating system. In place of these diaphragms there may be substituted diaphragmatic discs with apertures of progressive size, variable diaphragms, or absorbing glasses for increasing the measuring range of the instrument.

The axial ray of the incident beam of light formed by the source (S), lens (L), and diaphragms D1, D2, and D3 have been shown to make an angle of 90 degrees with the axial ray of Field 1 of the photometer (Ph). If it is desired to observe the scattered-reflected light produced by the turbid suspension in cell (C) at any angle other than 90 degrees with the incident beam of light, the opti-

* These apertures may be cut into cell (C) if the Tyndallmeter is to be adjusted to always observe the scattered-reflected light produced by the turbid suspension at a fixed angle with the incident beam of light.

cal system may be mounted so as to make the desired angle; or the optical system may be mounted on an arm which is rotatable about a point where the axial ray of the optical system producing the incident beam of light intersects the axial ray of Field 1 of the photometer as a center.

This work was originally undertaken for the design of a new instrument for the study of turbidity. It was soon found that it might easily be transformed into a colorimeter. It was concluded that the combined instrument would be more desirable since it would allow its use to be extended to a larger number of clinical laboratory determinations.

B. As a Colorimeter. The tyndallmeter may be transformed into a colorimeter by closing shutter 1(Sh1), thereby preventing the incident beam of light from entering cell (C), and opening shutter 2 (SH2) which permits another beam of light coming from the source (S) and falling upon the reflecting or diffusing surface plate (X) to enter cell (C). The path of the beam of light now entering cell (C) must be at a right angle with the incident beam of light previously described. Plate (X) is rotatable so that either diffuse or reflected light may be used to determine the absorption of the fluid placed in cell (C). D8 and D9 are diaphragms similar to those described for the tyndallmeter.

The same photometers used with the tyndallmeter may be used with the colorimeter. Colorimetry may be carried out by either of the two following methods.

1. A preparation of known strength of the solution to be studied is placed in cell (C′)† having two apertures and permitting the beam of light to pass through the solution. The solution of unknown strength is placed in cell (C). In each case the solution is contained in a test tube and the test tube is placed in the cell. The intensities of the two beams of light are now measured in the manner to be described in the next section.

2. Light filters (F1) and (F2) transmitting light in the region of one of the absorption bands of the solution to be studied are placed in the path of both beams of light as indicated in figure 2. The determination of the strength of the solution of unknown strength is now made by the measurement of the

† When the instrument is used for turbidimetry, the cell (C′) is not used except to permit a beam of light to pass through the two apertures, and the light filters (F1) and F2) are not in use.

"monochromatic" light absorbed by the solution in the region of the absorption band. The solution of known strength is no longer necessary for this measurement except for the calibration of the instrument. The properties of solutions used in clinical laboratory determinations are now being studied and suitable filters are being prepared.

II. The photometer cube and the photometer (figure 2)

The scattered-reflected light produced by the turbid suspension or the light absorbed by the solution in the test tube in cell (C) and the comparative source of illumination on plate (G) are brought into precise juxtaposition as two adjacent fields by means of the Duboscq‡ prism with the biprism occular (Ph), serving as a photometer cube. The brightness of the two fields is now adjusted to equality by means of the Nicol prisms (N1) and (N2). The Nicol (N1) is fixed and Nicol (N2) is rotatable. The scale is calibrated in quarter degrees of arc. The variation of the intensity (I) of field 2 is calculated from the formula $I = k \cos^2\phi$ where ϕ is the angle of rotation of Nicol (N2) measured in degrees of arc and k is a constant.

This arrangement is satisfactory in all colorimetric work. However, in turbidimetric work it is only satisfactory if the measurement of the scattered-reflected light is always made at any fixed angle with the incident beam of light. If this angle be varied and changes take place in the polarization of the light emitted from plate (G), the following precautions must be taken:

Plate (G) may be made of a substance which is a nearly perfect diffuser of light (a surface coated with magnesium oxide or magnesium carbonate, plaster of paris, or certain qualities of milk glass). Such a surface is necessary since perfectly diffuse light is unpolarized.

Another arrangement of the Nicol prisms offering a variety of measurement which is at times desirable may be mentioned. Nicol prisms (N2) and (N3) are adjusted so that their planes of maximum polarization are parallel to each other. If there is

‡ For this purpose the Lummer-Brodhun is the most desirable photometer cube, but it is more expensive. Any of the various forms of the Ritchie wedge described in Walsh[3], a Martens biprism[3], or a Bunsen[3] photometer are less expensive substitutes which may be used.

any polarized light emitted from plate (G), the common plane of the two Nicols must be adjusted parallel to the plane of symmetry of the polarized light emitted from plate (G). The variation of the intensity (I) in field 2 is now calculated from the for-

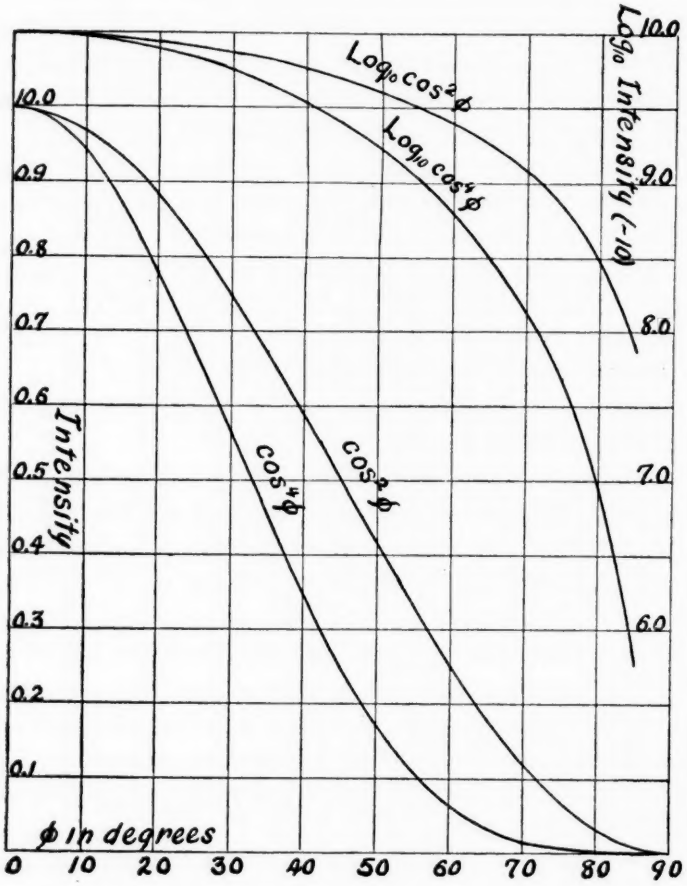

PLATE 1

mula $I = k \cos^4\phi$, where ϕ is the angle of rotation of Nicol (N1) measured in degrees of arc and k is a constant.

The relationships between the intensities expressed in terms of functions of $\cos^2\phi$, $\cos^4\phi$, $\text{Log}_{10} \cos^2\phi$, and $\text{Log}_{10} \cos^4\phi$ have been plotted in plate 1. The curves are of value in determining the

range of measurement with any one system of diaphragms and the precision with which measurements may be made in different regions of the scale around which the Nicol is rotated. The minimum precision in any region of the scale when the intensity is a function of $\cos^2\phi$ is better than 0.5 per cent. When the intensity is a function of $\cos^4\phi$, the minimum precision in any region of the scale is 0.7 per cent. If a logarithmic function is introduced, the rotation of the Nicol prism should not exceed 55° for $\text{Log}_{10}\cos^2\phi$ and 35° for $\text{Log}_{10}\cos^4\phi$, to obtain a precision of 0.5 per cent. Similar curves plotted on a larger scale are of great assistance in directly reading the intensity for each scale reading from the curve.

To obtain the precision stated in the preceding paragraph it is necessary to adjust the brightness of the photometric field so that a brightness level ranging from 20 to 30 millilamberts is secured. A more detailed description of the effect of "brightness level" upon the precision obtainable in photometry may be found in Lowry's[1] paper.

In place of the system of Nicol prisms one or more calibrated wedges may be substituted in one or both fields of the photometric system.

A Martens polarization photometer[2] may be substituted for both the system of Nicol prisms and the photometer cube.

III. The cell and test tubes (figures 3, 4 and 5)

The use of test tubes of varying size in the Tyndallmeter-Colorimeter has been made possible by observing a small constant area of the Tyndall cone produced by the scattered-reflected light in the turbid suspension. Figure 3 shows a square tube in cell (C) arranged for a turbidity determination. Screws K1 and K2 and their attached angle bars are for holding the test tube in position. The square tube is the ideal test tube to use for turbidity measurements as it permits the use of a path of light which traverses a very small distance, thereby eliminating many secondary effects which are produced when the path of light traverses a larger distance. Screw K2 is fixed and the size of the test tube will not affect the measurement provided the side of

the square tube is not smaller than the total length of the path of light in the test tube.

Round test tubes (figure 4) may be used in the Tyndallmeter-Colorimeter for turbidity measurements, but the path of light will have to traverse a longer distance if tubes of varying size are to be used. Large round test tubes varying in size as much as 4 mm. internal diameter may be used without correcting for the size of the tube. When Wasserman tubes are used, the permissable variation in size without correction is 1 mm. Figure 4 shows two round test tubes in place and the position of screw K2. By using a small beam of light which traverses a small distance in the test tube, the lens effect of the round tube does not enter into consideration as a variant for different size tubes.

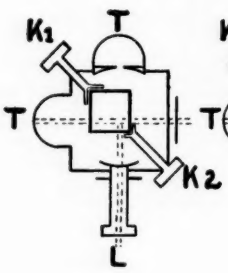

FIG. 3

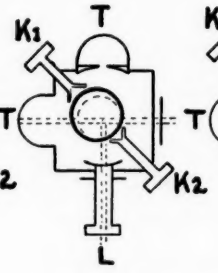

FIG. 4

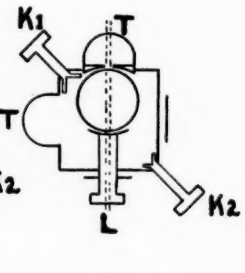

FIG. 5

Two hollow black tubes (T) sealed at one end are placed opposite each aperture in cell (C) to absorb the transmitted and reflected light.

Figure 5 shows a round test tube arranged for colorimetry. Screws K1 and K2 have been turned back and screw L now holds the test tube in position. The effect upon the measurement produced by using test tubes of different size may be calculated from Lambert's or Beer's laws for the absorption of light by fluids.

SUMMARY

A. Physical advantages of the Tyndallmeter-Colorimeter

(1) The precision of the Tyndallmeter-Colorimeter as experimentally determined by turbidity measurements with the Mar-

tens polarisation or the two Nicol prism photometer is 0.5 per cent.

(2) The turbidity or concentration of suspensions and solutions ranging in concentration from 1 to 10^4 may be determined.

(3) Turbidimetric and colorimetric measurements may be carried out in test tubes without removing the plug protecting the contents of the tube and without loss of any portion of the sample.

(4) Test tubes of varying size may be used in the instrument for these determinations. For turbidity work the size of the test tube will not affect the measurement. For colorimetry, the effect upon the measurement introduced by using test tubes varying in size may be calculated from Lambert's or Beer's laws for the absorption of light by fluids.

(5) One source of radiation (an electric lamp) is used for both the known and unknown substances, thereby automatically compensating for changes in the energy and other factors influencing the intensity or quality of the source of light.

(6) The standard of reference to which the turbidity or color of the unknown suspension or solution is compared is a physical unit which does not change with time nor deterioriate.

B. Applications of the Tyndallmeter-Colorimeter to measurements which are of interest in medicine

(1) The quantitative determination of the turbidity of all turbid substances.

(2) The determination of bacterial concentration in salt solution suspensions, as a function of the size and number of the organisms. Similar determinations may be made on broth cultures, but a correction must be made for the turbidity of the broth and the turbidity produced by the metabolic products of the bacteria.

(3) The determination of the average area of blood corpuscles when the number of corpuscles in a suspension is known. This value is of significance in anemic blood as an index of the average size of the red blood corpuscles if the number of white blood corpuscles is within normal limits.

C. As a colorimeter

(1) All colorimetric determinations such as blood sugar, blood urea, creatinine, and similar laboratory procedures can be performed.

(2) The hemoglobin content of blood can be determined.

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SHOULD THE PRECIPITATION TEST FOR SYPHILIS BE ADOPTED TO THE EXCLUSION OF COMPLEMENT-FIXATION PROCEDURES?*

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The advent of the Kahn precipitation tests for the laboratory diagnosis of syphilis unquestionably marked a new era in the re-valuation of various preëxisting tests for that disease. It has stimulated numerous valuable researches along the lines of "specificity" and "sensitiveness." Through such researches knowledge of the nature of the precipitation and of the complement-fixation reactions as applied to the laboratory diagnosis of syphilis has become considerably enriched. Indeed, the mechanism of these reactions can be expressed now in simple physico-chemical formulas. Investigators who make this subject their special study know what changes they may expect in the results following given changes in the reagents used.

A considerable number of such researches can be found scattered among the scientific and medical publications, while much of this information remains unpublished. Unfortunately many of the practical serologists read the published results entirely too casually, being content with abstracts or with the summaries of the original publications. It is safe to state that the majority of them regard such studies as of purely theoretical importance and are of the opinion that they have no practical significance or direct bearing upon the understanding of certain inconsistencies or discrepancies occurring in the results of the various tests for syphilis.

It is for this reason that the tube-precipitation test, for instance, because of its simplicity of manipulation, apparent definiteness

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of the standardization of antigen, comparative high degree of "sensitiveness" and "specificity" and facility of reading the results, has profoundly impressed the practical serologists in the United States and abroad. It has caused many of them to abolish the complement-fixation procedure and rely upon the results of the precipitation test exclusively. Enthusiasts went so far as to claim for their test superior, if not absolute "sensitiveness" and "specificity" and the attributes of a quantitative science.[2] The sero-luetic reports of the League of Nations Health Committee competitive conferences in Copenhagen and in Montevideo strengthened the impression that the complement-fixation procedures are by nature inferior and less reliable.[3] This despite the fact that at either of these conferences complement-fixation cold incubation procedures based upon the technical principles described by Kolmer[4] were not presented.

The above outlined situation has crystallized itself into a definite pressure upon serologists who still adhere to the use of the two types of procedures. This pressure is directed along several channels. First, there is the constant demand for "while you wait" results; second, there is the argument connected with the reduction in the cost; third, there is the altogether scientifically unjustifiable tendency to refer results of any and all tests to the tube-precipitation test. Even the best informed students of serology upon reporting a negative precipitation and positive complement-fixation (cold incubation) on the same serum specimen have been told that their complement-fixation technic yields nonspecific results, and is, therefore, confusing and useless; while if they report a reverse condition, they are told that their complement-fixation technic is insufficiently sensitive, and, therefore, again confusing and useless.

Serologists must take a definite well-founded stand as to whether one or two types of reactions shall be given preference in the practical serologic laboratory. The following brief discussion of some theoretical and practical aspects of complement-fixation and antigen precipitation is presented to assist in forming a rational opinion on this question.

It is well known that the precipitation test becomes positive

earlier than any complement-fixation test in some instances of early syphilis. On the other hand, I have reported consistently positive complement-fixation results in many instances of early syphilis in which the precipitation test lagged considerably. In this connection the old question arose as to whether the precipitins in the case of syphilis are identical or merely concomitant with the so-called lytic sensitizers. This question is of importance from a practical immunologic view-point. It is evident from the nature of the lytic sensitizers that the answer to this question cannot be attained without the assistance of the lytic agent per se-complement. Complement-fixation as a procedure, therefore, cannot be abolished from the practical serologic laboratory until this question finds its solution.

This argument in favor of complement-fixation may be regarded by many as a weak one. Indeed, if allowed to stand by itself it carries but little persuasion. Combined with the following arguments into a logical system of reasoning, it becomes significant.

From a physicochemical consideration any substance that is in solution is a part of the solution system, and any substance that has become precipitated from the solution is from a practical point of view without the solution system. In the instance of substances in the colloidal state this generalization is equally true, though in a somewhat qualified way. Generally speaking, the chemophysiologic influence of a substance in the colloidal state is determined by its chemical properties. However, many of its characteristics, such as the velocity and intensity of reaction, are determined largely by the enormous surface presented per unit mass. Where a mass-unit of substance is subdivided so that the resulting subdivision approximates "all surface," interfacial phenomena, such as surface tension, adsorption, electrical potential, and solubility become enchanced out of proportion to the mass relationships. Hence, the chemophysiologic reactivity of a substance in a high degree of subdivision may be as great as that of a substance in true solution. It may be regarded, therefore, with justice as within the solution system. Any physicochemical process, which reduces the surface area of the substance in extreme colloidal state to a point where visible pre-

cipitation and settling out appear, reduces its potency to a point nearing inertness. Hence, once the substance originally in colloidal suspension is precipitated, it can be regarded from a practical view-point as without the solution system.

This principle is of importance to the living organism teleologically. A substance which finds its way into the circulating fluid or into the fluids surrounding the tissues may be directly utilizable by the organism in its economy, or it may not be utilizable by the organism in its original state. If it is of the former type and is present in proportionate amount, it is of assistance to the organism in its normal physiologic processes. If, on the other hand, it is of the second type, it will hinder the physiologic processes of the organism and may be considered as toxic. It must be removed from the body.

If the molecules of the interfering substance, present in the body fluids in solution or in colloidal state, are of an organic nature and are such that they can be eliminated through the skin, respiratory or urinary tracts, or can be directly transformed into non-interfering molecules by the liver or other organ of the body, no toxic condition may arise and no immunologic defense reaction may be evoked. If, on the other hand, the molecules of the foreign organic substance cannot be rendered harmless by the means just enumerated, in most cases immunity reactions, as understood by serologists, come into play. The injurious substance must be placed without the solution system of the body as the first and immediate step towards protection. To accomplish such precipitation, the immunoprecipitins are generated by the living organism. These immunobodies undoubtedly serve other purposes with which this discussion is not concerned. The earlier the precipitins appear and the more generous their amount, the greater the body protection.

Once the foreign substance has become precipitated, its immediate injurious effect to the organism is removed. The lytic sensitizers then may come into play and sensitize the substance to the action of complement. The latter, whatever its mechanism of action may be, transforms the original substance, so that the resulting molecules can either be eliminated or utilized by the organism.

If this teleologic theory of the process of immunity is accepted, then it must be assumed that precipitins appear first, and, therefore, can be first demonstrated. It is possible that this may well answer the question as to why in a large percentage of cases of beginning syphilis positive precipitation results are obtainable comparatively early. It may constitute a rational scientific reason for the use of the precipitation test for syphilis. It cannot, however, be regarded as an argument in favor of the abolition of the complement-fixation tests, since other factors arising from the quantitative relationship of the immunologic principles must be considered.

The well known adage of practical medicine "every case is a law unto itself" is applicable to immunologic manifestations in vivo and in vitro. Unquestionably, this is largely responsible for the failures in generalized therapeutic immunology. The factors entering into play are numerous and variable, and at the present stage of our knowledge many of them are unaccountable. If we assume, according to the above outlined theory, that the precipitins and sensitizing lysins constitute two distinct factors and that the precipitins, as a matter of immediate protection, are generated by the living organism in advance of the sensitizing lysins, we still remain in ignorance with regard to the progress of their generation in any individual case.

What, for instance, would be the effect upon the results of antigen precipitation and complement-fixation in a case in which the generation of the precipitin is slow, though early, and that of the lytic sensitizer is normal or accelerated? Here, the factors of dispersion, of reactive surface area of the suspended particles, and the manner in which complement combines with the sensitized suspension, offer a possible explanation. The recording of the tube-precipitation results, it must be remembered, depends upon ocular observation. But ocular visibility has its limits even with the aid of a magnifying glass. Where the concentration of the precipitin is low, high dispersion will result. High dispersion means an increased total surface area of the particles suspended. With the normal or accelerated rate of generation of the lytic sensitizer, enough of it will be present to sensitize the entire surface area of the highly dispersed suspensoid.

It is now generally conceded, in fact it is nigh well proven, that complement acts through the process of adhesion by coating the surface area of the sensitized particles in the suspensoid in a layer of definite effective thickness.[1,5] The greater the surface area of the sensitized substance, the greater the consumption of complement. Hence from a practical laboratory viewpoint in the above hypothetical case there is a comparatively small total volume of an immunologically sensitized substance, so highly dispersed as to be invisible to the eye alone or with the aid of a magnifying glass. The result, therefore, must be reported as negative by the usual tube-precipitation method. Though beyond the visibility of the eye, the suspensoid represents a truly immunologically sensitized complex, since the quantity of lytic sensitizer was assumed to be generated at a progressively greater rate than the precipitin. It is, therefore, capable of adsorbing complement according to the accepted principles of immunology. This substance, being highly dispersed and, thereby, forming a large surface area, is capable of fixing a complete dose, or more, of complement. The result is a four plus complement-fixation, in the presence of a negative precipitation.

From the antigen-precipitation negative to the doubtful is only a short step. The Kahn doubtful, according to the author of the test, is to be reported as negative. Yet, the occurrence of serum specimens giving results of Kahn tests which for research record purposes were marked as "doubtful-trace in one or two tubes" and which resulted in strongly positive complement fixation tests have not been infrequent in my experience. True enough, they occur very infrequently in cases of early syphilis and most frequently in treated cases. But in this very fact I see a possible danger. In the absence of a better criterion for the treatment of syphilis, many syphilologists have been guided by the laboratory results. Much, of course, has been said and written to condemn such a practice. Since, however, nothing even approaching a positive substitute has been offered, the practice has been continued by some of our best syphilologists. The use of both the precipitation and complement-fixation tests eliminates the possibility of making the undesirable impression upon the

clinician. By receiving precipitation negative and complement-fixation positive reports, the clinician will have his attention directed to the fact that antibody is still detectable by one of the tests used. Thereby, the laboratory will have fulfilled its function.

Further theoretical considerations of the mechanism of the tube-precipitation test and experimental evidence strengthen the objection to the elimination of the complement-fixation procedure from the practical laboratory. It was shown by many investigators[8] that up to the addition of the hemolytic system, the complement-fixation and antigen-precipitation tests are effected by the same immunochemical mechanism. However, in the first case, an indicator must be added to make the end result evident, while in the latter the end result becomes directly visible to a varying degree. Such visibility is brought about by purely mechanical means, based upon certain physicochemical properties inherent to the colloidal suspensions primarily concerned in the tests.

Upon the addition of the serum to the antigenic suspension employed in either the antigen-precipitation or the complement-fixation procedures, active suspended nuclei are formed. A complexity of interfacial forces arises at the surfaces of these nuclei. A particular combination of these forces exerts a selective attraction upon the precipitins and lytic sensitizers. Through the process of adsorption the latter coat the suspended particles in a successive order and become denaturized through the process of dehydration, polymerization, or the like.[9] At the same time the difference between the surface tension of the medium and the suspended particles of the antigen is altered. The surface tension of the new sensitized antigenic particles in particular acquires an optimal value which makes the discrete particles susceptible to complete or partial aggregation and coalescence.

The suspended particles are in constant molecular motion and frequently collide. If the frequency of the collision and the impact force are great enough to overcome the resultant of the forces of dispersion, the sensitized particles will aggregate and coalesce forming progressively increasing spheres or spheroids. At

a certain point the particles become too great and too massive for the Brownian movement. Further collision and coalescence will cease. It follows, that the greater the concentration of the suspensoid, up to a proper colloidal balance, the greater is the opportunity for the particles to collide and coalesce; also, the greater the concentration of the sensitizer, the greater the favorable change in the surface tension, and, hence, less impact force is required to effect coalescence. Other factors, such as the influence of the concentration and of the type of electrolyte present undoubtedly are of equal importance.

In the case of the complement-fixation technic all the reagents are used in high dilution. The number of spheres or spheroids per unit volume is comparatively small, the distance between the particles is comparatively great, while the amount of precipitin and, hence, the reduction in the surface area and the optimal change in the surface tension of the particles are comparatively small. It is evident, therefore, from a simple consideration of dynamics, why the particles should remain invisible and why the indicator should be necessary to make the end result demonstrable.

In the case of the precipitation test all the reagents are used in the greatest possible concentration, and the final volume is kept at a minimum. This makes the distance between the particles short. The particles per unit volume are numerous. The changes in the surface tension difference and in the surface tension of the sensitized particles per se are shifted to magnitudes more favorable than in the case of the reagents used in the complement-fixation test. This leads to an increase in the frequency of collision between the particles and to a greater susceptibility on their part to coalescence. It makes the same impact force, due to molecular movement of the particles, more effective in causing the aggregation and coalescence of the sensitized particles. Hence, the visibility of the results of the tube-precipitation test.

However, even in the Kahn test the particles soon reach a size too large for the force supplied by the Brownian movement. Depending upon the balance of forces, aggregation and coalescence of the particles stop when the so-called precipitate is

barely or not at all visible in many positive serums. An outside force must be adopted; hence, the shaking process which continues with greater force and greater effectiveness, the work started by the Brownian movement of the particles. The process is apparently similar to that of churning, but too forceful and too long continued shaking may reëmulsify the aggregated and coalesced particles in some positive serums. Therefore, the time of shaking and the number of agitations per minute must be prescribed.

This definitely prescribed time and manner of shaking is an essential factor from a practical consideration. But it carries with it its own weakness, and presents further argument against the adoption of any precipitation test to the complete exclusion of a well standardized cold incubation complement-fixation test. As in clinical medicine "every case is a law unto itself," so in serology "each serum has its own idiosyncracies." The force supplied by the shaking process may be sufficient to bring about maximal visibility in one specimen, it may be insufficient for another, and may be too much, and hence, have a dispersive effect on still another. From a practical laboratory consideration, therefore, it is evident why some Kahn tests should read negative or doubtful (reportable negative), yet the complement-fixation tests should be positive in varying degree. This has occurred repeatedly in my experience with serums from individuals with definite histories of syphilis. The fact that some of them may be treated patients, or patients who have received maximal treatment, or patients who may be considered by some syphilologists as cured, matters not so far as the thesis of this paper is concerned. No agreement exists among the best syphilologists upon the definition of cured or even arrested syphilis; nor is it definitely established whether the antibody demonstrable in a long treated case of syphilis is an indication of continued immunity or of a feebly continued or reawakened activity of a host. What matters, indeed, is the proper decision as to whether serologists should rely upon ocular visibility of precipitation tests alone as a scientific basis for their serologic judgment, setting aside once and for all complement-fixation procedures as a

practical laboratory application. In the light of this discussion, the answer is unequivocally negative.

EXPERIMENTAL

If the shaking process used in the Kahn test is insufficient for some serums and redispersive for others, cannot an applicable mechanical force be resorted to which would prove more general in its efficacy as a coalescing agent? Indeed, such a force is supplied by centrifugation. It has been employed by investigators previously and by myself in some of my[7] theoretical studies. Recently Mueller[10] applied it to his laboratory test for syphilis. The following experiments prove that some Kahn negative and doubtful tests contain immunologically sensitized reagin in an invisible state of division.

One series of cholesterolized and one of Kolmer lecitholized antigen complement-fixation tests and two sets of Kahn tests were prepared on a number of the same serums. One Kahn series was completed, according to Kahn routine, using the second and third tubes only.[6] The results were recorded for each tube. The duplicate series was placed in the incubator for one hour, following the shaking. At the end of one hour and prior to the addition of the saline the tubes were centrifugalized for ten minutes at a speed exceeding 3000 revolutions per minute. One half cubic centimeter of saline was then added to each tube. According to the completeness with which the antigen became removed from suspension and the degree of coalescence of the suspended particles, the results were recorded as usual, ++++, +++, ++, +, and doubtful. This experimental procedure was applied to one thousand random specimens. Some of the results which have a direct bearing on the subject under discussion are tabulated.

The cholesterolized and the Kolmer are three-tube cold incubation tests. In the table the control tubes are not reported, since tests with anticomplementary results were omitted. After recording the results of each tube, the Kahn-experimental series were thoroughly shaken and carried through a complement-fixation procedure as described by me[7] in another paper.[8] In every case the results were positive. An equal number of confirmed negative tests were subjected to a similar procedure. The complement-fixation results in every case were negative. The presence of a highly dispersed sensitized reagin, invisible to the standard Kahn test has been demonstrated by this experiment in every case where one or both of the complement-fixation tests were positive. The significance of the few cases in which all the routine tests were negative and the post-centrifugalized positive has no direct bearing on the subject under discussion and will be discussed elsewhere.

The results of the tests recorded in the table were taken at random from the research notebook. No information was appended to the records bearing upon the clinical histories of the cases. It might have been suspected, therefore, that in some of the cases there was no history of syphilis. Were it so, the results

TABLE 1

COMPARISON OF RESULTS BY DIFFERENT METHODS

NUMBER	CHOLESTEROLIZED	KOLMER	KAHN	EXPERIMENTAL
1	2.1	1.1	0.0	2.3
2	4.4	3.1	Tr. Tr.	2.3
3	4.4	4.4	Tr. Tr.	3.3
4	3.1	0.0	0.0	0.0
5	4.2	3.2	0. Tr.	1.2
6	0.0	0.0	0.0	1.1
7	2.1	0.0	Tr. Tr.	2.3
8	3.2	1.0	Tr. Tr.	3.4
9	3.2	2.0	0.0	4.4
10	2.1	0.0	0.0	3.4
11	4.4	0.0	Tr. Tr.	4.4
12	0.0	0.0	0.0	2.2
13	4.4	1.1	0.0	1.2
14	2.1	1.1	0.1	4.4
15	4.4	3.3	0.0	2.2
16	3.2	0.0	0.0	2.3
17	3.3	3.3	0.0	1.1
18	4.4	4.4	0.0	1.0
19	0.0	0.0	0.0	3.4
20	3.3	3.2	0.0	2.2
21	3.1	1.0	0.0	4.4
22	[illegible]3	2.2	0.0	2.2
23	[illegible]3	2.1	0.0	3.4
24	4.4	4.4	0. Tr.	1.1
25	2.1	1.1	0.0	3.3
26	1.0	2.1	0.0	2.2

of the experiment would be valueless. However, after the table has been compiled, excerpts were made from the clinical records of the cases. In each there was a history of syphilis. Some of the excerpts are presented below:

(1) In June 1930, the patient observed an ulcer on the vulva which persisted several days. July 31, 1930, blood Wassermann (type not described) was ++++. Had received treatment. Admission Wassermann (warm incuba-

tion) Negative; Kahn, ++++. Diagnosis: Syphilis, secondary, recurrent. Patient is under treatment. History of serology: July 2, 1931, Cholesterolized and Kolmer, Doubtful; Kahn, +; August 23, 1931, all tests ++++; December 11, 1931, as shown in table.

(2) Patient complains of vaginal discharge and pain in lower abdomen. History of miscarriage, claimed to be accidental. States blood tests taken one year prior to this examination was Negative. Upon admission serologic tests were: Cholesterolized, ++; Kolmer, ++; Kahn, +. December 11, Cholesterolized, +++; Kolmer, ++; Kahn, Negative.

(3) Diagnosis: Syphilis, latent. Feb. 5, 1929, Wassermann (warm incubation) and Kahn, Negative. Several months later, Wassermann, and Kahn, +++. September 3, 1931, Wassermann and Kahn, Negative. December 11, 1931, Cholesterolized, +++; Kolmer, ++; Kahn, Negative.

(4) Diagnosis: Syphilis, primary. History of serology: March 30, 1931, Wassermann (warm incubation) Negative; Kahn, ++++. August 7, 1931, Cholesterolized, ++++; Kolmer and Kahn, +. December 7, 1931, all tests Negative.

(5) Syphilis, secondary. History of treatment.

(6) Had genital lesion twenty years ago. Antisyphilitic treatment one year ago. Serology vascillated throughout the period of observation from Negative, to strongly Positive, then Doubtful, and again strongly Positive, the Kahn test being Negative most of the time. December 11, all routine tests were Negative, while experimental test was ++++.

(7) Had sore on penis two years ago. Spinal tests all positive. Diagnosis: Neurosyphilis. It is worth observing that in this case on December 7, 1931, the serologic reports were: Cholesterolized, ++++; Kolmer, ++++; Kahn, Negative; Experimental, +.

(8) Diagnosis: Syphilis, latent. Contracted eight months ago. Only complaint upon examination: itching of skin. From March 23, 1931 to November 2, 1931, serology was consistently Negative. On that date, Cholesterolized, Kolmer, and Kahn, ++. November 9, 1931, Cholesterolized, ++; Kahn and Kolmer, Doubtful. December 10, 1931, all tests were Negative, while experimental was ++.

(9) Patient was referred by Mexican Health Center with positive serology. Diagnosis: Syphilis, Latent.

(10) Patient is five months pregnant. Three weeks prior to presentation at Clinic had Kahn, ++++. Is free from clinical symptoms. Upon examination Cholesterolized, Kolmer and Kahn were ++++. September 28, 1931, all tests were again ++++. December 7, 1931, Cholesterolized, ++; Kolmer, Negative; Kahn, Faint trace; experimental, ++.

(11) Had syphilis in 1920 at which time patient received treatment. Has been free from clinical symptoms for six years, and none were observable at the time of presentation at the clinic. But serologic tests were: Wassermann (warm incubation), ++; Kahn, ++++. May 18, 1931, Wassermann (warm

incubation), +++; Kahn, ++++. August 25, 1931, Cholesterolized, ++; Kahn, ++++. December 7, 1931, Cholesterolized, ++; Kolmer, Doubtful; Kahn, Faint trace; Experimental, ++++.

(12) Diagnosis: Syphilis, latent, neurosyphilis. Serology varied from fixation positive, precipitation negative, to reverse. Cerebrospinal fluid tests, Positive.

The twelve excerpts are like the remaining fourteen. The points of importance brought out by excerpts are: (1) In treated cases of syphilis both types of tests show periods of nonreaction. In most cases these periods are not coincidental. (2) No parallelism exists between the results of the precipitation or complement-fixation tests and the clinical symptoms. (3) The serologic results of either of the two types of tests follow no well defined curve of intensity-reduction in the course of treatment. (4) Where the standard Kahn is negative in treated cases, the lecitholised Kolmer is frequently positive, (the reverse is also true to a great extent, but we are not concerned with this in the present paper), the cholesterolized is generally positive, and the experimental centrifugalization results are nearly always positive. The results of the experiments, as summarized in the preceding table, supported by the information supplied by the excerpts from the histories, strengthen the thesis of this paper and offer the concluding argument against the abolition of the standardized complement-fixation procedures from the diagnostic laboratory and the adoption of the precipitation test as the sole demonstrator of the presence of reactive substance in syphilitic serum.

SUMMARY

Definite pressure has been brought to bear upon workers in the field of serology in syphilis to eliminate the complement-fixation procedure from the diagnostic laboratory, and to limit the serologic diagnosis of syphilis to the tube-precipitation test. To assist serologists and laboratory technologists in deciding upon the stand to be taken in relation to such pressure, a general discussion of the principles upon which precipitation and complement-fixation tests are based is presented. The bearing which the dualistic conception of the origin of luetic precipitins and lytic

sensitizers may have upon the interpretation of the discrepancies occurring between the results of the precipitation and complement-fixation results is also discussed. The term lytic sensitizers instead of lysins is here used advisedly. A teleologic theory of the functions of the precipitins and lytic sensitizers is presented. In the light of this theory it is assumed that precipitins are generated in advance of the lytic sensitizers. It is further assumed that the progress of generating precipitins may be less, parallel to, or greater than that of the lytic sensitizers. Conjointly with the mode of the complement function such progress variation, it is pointed out, may have a direct bearing upon the differences in the results of antigen precipitation and complement-fixation. Experimental evidence is presented to prove on a practical basis the points brought out in the discussion of the physicochemical factors which in the same serum may be favorable to a strong complement fixation and unfavorable to the tube-precipitation test. It is concluded that nonvisibility of the precipitate in the tube-test does not constitute a scientific criterion for the judgment as to the absence of the immunologically sensitized antigen-antibody complex. A well standardized and properly carried out complement-fixation test demonstrates the presence of sensitized complexes in most positive serums, no matter how high the degree of their subdivision may be. It is, therefore, concluded that the complement-fixation, including overnight icebox incubation and the tube-precipitation tests, must be continued as mutually supplementary adjunct procedures to the diagnosis and control of syphilis.

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VALUE OF H AND O AGGLUTINATION IN DIAGNOSIS OF TYPHOID*

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Since the advent of prophylactic vaccination against typhoid fever, the interpretation of agglutination reactions has been confusing. This is due to the fact that the serum of inoculated persons will agglutinate the organism in question. In view of this fact Dreyer[2] introduced his method of repeated estimation of the agglutinin content of the patient's serum. However, in the case of *Salmonella paratyphi* (*B. paratyphosus A*), the agglutinin response may be so slight as to render the repeated estimations uncertain. Recently, Felix[3] published evidence to show that protective inoculations lead to the formation of large flaking agglutinins, while the agglutination in typhoid fever and in a non-inoculated person is, without exception, of the small flake type.

The explanation of the small and large flaking forms is found in the work of Weil and Felix[9] who showed that Proteus X exists in two forms (O and H forms), which differ from each other morphologically, biologically and serologically. They represent two forms of variation mutually transferable into each other. The anti-O-serum contains one agglutinin which reacts specifically with the homologous organisms agglutinating them in small flakes (O-agglutination); antiserum against the H type contains two agglutinins, the specific small flaking O and a nonspecific large flaking H which reacts with heterologous as well as with homologous organisms. The O receptors are thermostabile while the H receptors are thermolabile (Sachs).[8]

Burnet[1] believed that the O types are due to the somatic proteins, and the H type to the flagellar proteins. Accordingly,

in a given case of typhoid fever there is an overwhelming stimulation of the O or small flaking agglutinins, while in the inoculated person the H type or large flaking agglutinin predominates. In a series of eighty-seven individuals given antityphoid inoculations, Gardner[5] observed the production of the O form, and pointed out that it is likely inoculation does not cause, on an average, nearly so great a rise of the O forms as does typhoid fever, and therefore, the two conditions are not readily distinguishable. The O titre is higher shortly after inoculations than after a long period has past.

The question of a difference in the type of agglutination produced between the serum of a person actively infected and one previously inoculated is not a closed one. The recent outbreak of typhoid fever in the Cleveland State Hospital for the insane afforded an opportunity to investigate this problem further.

TECHNIC

The method of Felix was followed in detail. The strain of typhoid bacillus was the Kinyoun strain of the Ohio State Department of Health. The organism does not self-agglutinate. Twenty-four hour growths on plain agar were washed off with 1.5 to 3 cc. of saline depending on the size of the slant, and one drop of the suspension was added to each tube of serum dilution (1 cc.). The following dilutions were used: 1:50, 1:100, 1:250, 1:500, 1:1000 and 1:2000, and the usual saline organism controls. The tubes were incubated at 37°C. for two hours and sixteen hours at room temperature. All observations were made with a magnifying glass and readings were also made at the end of forty-eight hours. Both inactivated and active sera were also employed.

RESULTS

In a series of forty-two typical cases of typhoid fever it was found after eighteen hours that in a 1:50 dilution 90.2 per cent showed small flakes and 7.3 per cent large flakes. At the end of forty-eight hours the percentage showing the small flake type dropped to 70.7 per cent, while the large flake type increased 26.8 per cent. (See table 1.)

In the 1:100 dilution at eighteen hours the small flaked agglutination was 80.5 per cent, dropping to 75.6 per cent in forty-

eight hours, while the large flake type increased from 9.7 per cent to 17.1 per cent.

In a series of forty inoculated individuals including six treated intravenously for shock therapy, seven vaccinated from sixteen to eighteen months before, four vaccinated five months previously, and twenty-three from four to six weeks before, it was observed at eighteen hours that in a dilution 1:50, 55 per cent of the serums showed small flakes and 37.5 per cent large flakes.

TABLE 1

THE PERCENTAGE OF SMALL AND LARGE FLAKE AGGLUTINATIONS WITH SERUMS OF CASES OF TYPHOID FEVER

ACTIVE CASES		SMALL	LARGE	MIXED	NEGATIVE
dilution	*hours incubated*	*per cent*	*per cent*	*per cent*	*per cent*
1:50	18	90.2	7.3	0	2.4
	48	70.7	26.8	2.4	0
1:100	18	80.5	9.7	0	9.7
	48	75.6	17.1	2.4	4.9

TABLE 2

THE PERCENTAGE OF SMALL AND LARGE FLAKE AGGLUTINATIONS WITH SERUMS OF INOCULATED PERSONS

IMMUNE CASES		SMALL	LARGE	MIXED	NEGATIVE
dilution	*hours incubated*	*per cent*	*per cent*	*per cent*	*per cent*
1:50	18	55.0	37.5	2.5	5.0
	48	40.0	52.5	2.5	5.0
1:100	18	42.5	42.5	0	15.0
	48	47.5	40.0	2.5	10.0

In forty-eight hours the ratio had changed to 40.0 per cent for the small flake type and 52.5 per cent for the large flake type. At eighteen hours in the 1:100 dilution 42.5 per cent were of the small and 42.5 per cent of the large flake type. In forty-eight hours 47.5 per cent showed small and 40.0 per cent large flakes. (See table 2.) This ratio was not appreciably different between those vaccinated recently and those vaccinated eighteen months previously, although the serums of those individuals vaccinated recently showed agglutination in higher titers.

Although it is thus shown that 40 to 50 per cent of the serums of inoculated individuals agglutinated in small flakes when classed as to size, in many of these cases the floccules appeared to be less compact and more loosely scattered than those of the same size in the serums of patients with typhoid fever, in which the agglutination appeared more compact and granular in type.

Felix believed that it was necessary to use only a single serum dilution and suggested the 1:100 dilution. We found, however, that a single dilution could not be relied upon for representative results. The higher dilutions used by us showed nothing of importance. Gardner[5] used both 1:100 and 1:400 dilutions.

Our results demonstrate that while there is a predominance of O-agglutination in the serums of those actively infected with typhoid there is also O-agglutination in about 50 per cent of those inoculated against the disease, regardless of time whether recently or long before.

Felix and Olitzki[4] pointed out that with *Eberthella typhi* (B. typhosus), *Salmonella paratyphi, Salmonella schotmülleri* (B. paratyphosus B), and *Salmonella enteritidis* (B. enteritidis), low concentrations of phenol and formol produced definite inhibition of O (small flake) agglutination, while the H (large flake) type was unaltered. Alcohol in low concentrations seemed to produce no such inhibitory effect, but in high concentrations alcohol inhibited the H type of flocculation. In a series of ten serums from inoculated persons and fifteen serums from active cases the agglutination with live organisms was compared with that produced in bacterial suspensions made with 0.1 per cent formol, and 5 and 50 per cent alcohol. With the formolized suspensions there was 70 to 100 per cent H flocculation, the average being 76.6 per cent (see table 3). With 5 per cent alcohol suspension of bacteria there was also a predominance of the H type, 20 to 90 per cent (see table 3), although with a much lower average (60.0 per cent). Fifty per cent alcohol completely inhibited the H agglutination, there being about 50 per cent 0 and 50 per cent negative results (see table 3). This bears out Felix' contention that the use of live bacteria is essential for a clear differentiation.

THE "ANAMNESTIC REACTION"

Krauss and Barrera[6] reported two cases of a positive Weil-Felix reaction (Proteus OX_{19}) in a case of typhoid and a case of measles. In the case of typhoid the Weil-Felix was positive

TABLE 3

INCIDENCE OF DIFFERENT TYPES OF AGGLUTINATION OF TYPHOID BACILLI, SHOWING THE EFFECT OF FORMOL AND ALCOHOL

TEST		LIVE ORGANISMS			0.1 PER CENT FORMALIN			5 PER CENT ALCOHOL			50 PER CENT ALCOHOL		
		Small	Large	Negative	Small	Large	Negative	Small	Large	Negative	Small	Large	Negative
(1) Serums of fifteen active cases													
dilution	*hours incubation*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1:50	18	80	20	0	20	80	0	10	90	0	20	0	80
	48	70	30	0	0	100	0	10	90	0	30	0	70
1:100	18	70	30	0	70	30	0	60	40	0	10	0	90
	48	70	30	0	30	70	0	60	40	0	10	0	90
(2) Serums of ten inoculated individuals													
1:50	18	93	0	7	20	80	0	20	80	0	53	0	47
	48	67	33	0	20	80	0	40	60	0	53	0	47
1:100	18	73	7	20	20	80	0	40	60	0	20	0	80
	48	67	20	13	7	93	0	80	20	0	33	0	67

TABLE 4

SHOWING AGGLUTINATION WITH PROTEUS OX_{19}

AGGLUTINATION WITH OX_{19}		IMMUNE	ACTIVE
		per cent	*per cent*
2 hours incubation	Positive	57.1	78.9
	Negative	42.8	21.0
18 hours incubation	Positive	100.0	100.0

before a positive Widal of 1:4000 was obtained. Both patients lived in Buenos Aires where typhus fever is not known. However, one came from Russia and one from Ireland where endemic

typhus fever exists. More recently Palacios and Armijo[7] obtained positive Weil-Felix reactions in twelve cases of typhoid fever. The reaction was positive in dilutions of from 100 to 200 but in Chile, typhus fever is endemic.

We tested all serums from both the active cases of typhoid fever and the inoculated individuals using dilutions of 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320. After two hours at 37°C., the serums from the inoculated persons agglutinated (O type) in 57.1 per cent of the tubes, and no agglutination was seen in 42.8 per cent (see table 4). The active typhoid cases showed O type agglutinations in 78.9 per cent and no agglutination in 21.0 per cent. After standing at room temperature for an additional eighteen hours all the serum agglutinated. All the serum agglutinated in dilutions up to but not higher than 1:80. We believe that the reaction should be interpreted on the basis of nonspecific stimulation.

CONCLUSIONS

(1) Agglutination reactions were performed by the method of Felix with the serums of forty persons inoculated against typhoid fever and with the serums of forty-two patients with typhoid fever.

(2) Large flaked or H type agglutinations occurred in 40 to 52.5 per cent of serums of inoculated individuals. The small flaked or O type was noted in 70 to 90 per cent of the serums from typical cases of typhoid.

(3) While the H type of agglutination, in most instances, would point to inoculation as the cause, the O type of agglutination cannot be relied upon to designate active typhoid infection.

(4) Formalin, 0.1 per cent, inhibits the O type to a large extent, and 5 per cent alcohol to a lesser extent.

(5) A Weil-Felix reaction was present in the cases studied as evidenced by agglutination of the Proteus OX_{19}.

We wish to express our thanks to Dr. Guy Williams of the Cleveland State Hospital of the Insane for the opportunity to make the study and also to Doctors C. S. Sandhu and G. Little of the same institution.

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SERODIAGNOSIS OF MALIGNANT DISEASE

Preliminary Report

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Numerous attempts have been made to develop a satisfactory test for malignancy using blood serum. These attempts have been made along three principle lines, namely, chemical complement-fixation tests, and precipitation reactions. As new as the subject of diagnosis of malignancy by a study of the blood may seem, a full review of all the methods advocated would in itself be of burdensome length. Studies upon this subject were begun about twenty years ago by M. Ascoli of Italy. Of the chemical tests may be mentioned the meiostagmin reaction of Ascoli and Izar, the work of Freund-Kaminer, Ruffo, Bothello, Kahn (Germany) Abderhalden, Shaw-Mackenzie, Fuchs and others. All of these have proved unsatisfactory in other hands.

After an extensive investigation, Fry[1] devised a flocculation test for cancer which he used extensively in the Cancer Research Hospital, London. Landau started his studies on a precipitation test for malignancy in 1928 and in a series of 767 demonstrated carcinoma cases, 75.3 per cent gave a positive reaction, and in 826 proved cases of sarcoma, 75.5 per cent gave a positive result.

L. Hirszfeld and W. Halber[2] have described a complement-fixation test for malignancy.

In 1929 one of us demonstrated a certain affinity of an alcoholic extract of carcinomatous tissue for the serum of patients having malignant tumors. After modifications of our technique, a series of nearly three hundred cases has given results which are sufficiently encouraging to warrant further intensive study. While complete accuracy has not been demonstrated, it must be

borne in mind that none of the serological tests for syphilis, now twenty-five years old, is absolutely accurate.

METHOD OF PROCEDURE

(1) The serum is drawn in the same way as for any other serological test. Only raw serum is used which is kept in the icebox at 8 to 10°C.

(2) For an antigen, the tissue from a malignant tumor, removed surgically or at autopsy, is dissected carefully free from all fat and normal tissue and ground. To every gram of ground moist tissue so prepared 4 to 10 cc. of 95 per cent alcohol is added. If the quantity of tissue permits, several proportions of tissue to alcohol are used. This mixture should stand eight to ten days in the incubator or icebox. Good results have been obtained from both ways. Not

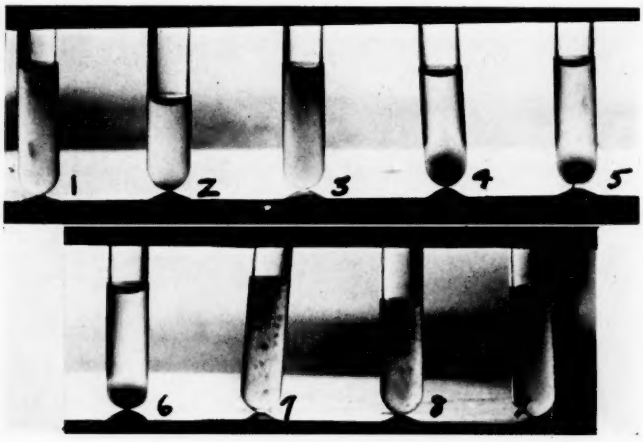

FIG. 1. TUBES 1, 2 AND 3 SHOW NEGATIVE TESTS; TUBES 4, 5 AND 6 SHOW POSITIVE TESTS; TUBES 7, 8 AND 9 SHOW NEGATIVE TESTS WITH CLUMPS

every tissue, however, produces a good antigen in all extract proportions and each one must be tried and titrated with positive and negative controls, and may have to be discarded.

(3) Nine tenths per cent saline is used.

(4) Two hundredths cubic centimeters of raw serum is pipetted into tubes 8 by 50 mm. To the serum 0.5 cc. saline is added, the test-tube rack shaken and 0.1 cc. to 0.2 cc. antigen (depending on the titration) is added. Again the rack is shaken and placed in the icebox at about 8 to 10°C. for four to six hours, sometimes longer. It is desirable to have five controls, negative and positive. The reaction must be watched until the control positives (see fig. 1, tubes 4, 5, 6) show a sedimentation with clear supernatant fluid. The negative tubes are cloudy and colloidal (see fig. 1, tubes 1, 2, 3) without sediment. Sometimes one can see in the negatives suspended clumps, (see fig. 1, tubes 7, 8, 9).

Table 1 shows the results so far obtained.

We are unable to explain why all cancer cases did not give a positive serological test. It must be remembered that some of these malignant neoplasms were young and small, such as carcinomas of lip and cervix and these possibly had not yet become a sufficiently systemic disease as to produce reactive substances in

TABLE 1

	CASES	POSITIVE		DOUBTFUL		NEGATIVE	
		Cases	Per cent	Cases	Per cent	Cases	Per cent
Blood taken preoperatively, diagnosis of malignancy confirmed by microscopical examination	43	38	88.3	1	2.3	4	9.3
Blood taken postoperatively, diagnosis of malignancy confirmed by microscopical examination	33	17	51.0	10	30.3	6	18.1
Clinical diagnosis of no malignancy; confirmed by microscopical examination	28	3	10.0	6	21.4	19	67.8
Clinical diagnosis of malignancy; no microscopical examination	126	92	82.8	11	4.5	23	12.6
Malignancy suspected but not definitely proved	59	17	28.8	7	11.8	35	59.0
Diagnosis: Normal pregnancy	70	4	5.9	8	11.9	58	82.0
Diagnosis: Tuberculosis	23	8	34.7	4	17.3	11	47.8
Diagnosis: Serologically positive syphilis	13	2	15.3	4	30.7	7	53.8

the blood stream. In postoperative cases, only 51 per cent gave positive reactions. A similar observation was made by Volkman.[3]

Syphilitic serums gave 15 per cent positive reactions. Landsteiner and his coworkers showed (1907) that extracts from all normal tissues gave positive results with syphilitic serums. In dissecting malignant tumors, it is impossible to remove all traces of normal tissue since a certain amount of fairly normal stroma is produced in any tumor growth and also bits of normal tissue are found in any area which a malignant tumor is invading or replacing.

Tuberculosis gave 24 per cent positive reactions. At the present time no satisfactory explanation can be given for this fact. It can only be surmised that a similar reactive substance is produced in the blood in both conditions. E. Witebsky[4] said that the right to speak about a specific serologic change in cancer is only possible if the control serums, tuberculosis and pregnancy, do not give a positive reaction. But still he thought there were signs that spoke for specific changes in cancer.

In normal pregnancy, 6 per cent gave positive reactions. It is to be remembered, however, that in this condition a false positive syphilitic reaction is occasionally found. One worker found that a few serums from pregnant women gave a positive complement-fixation reaction with alcohol as an antigen.

SUMMARY AND CONCLUSION

A certain affinity between an extract of malignant tumor tissue and the serum of patients having malignant tumors produces reactions.

We have presented a test for malignancy which in our hands gives about 90 per cent accuracy in cases with malignant tumors. The occurrence of positive reactions in tuberculosis, syphilis and in pregnant patients although not satisfactorily explained, does not seriously interfere with the diagnostic purposes in this test.

A large volume of material with good clinical histories and pathological control would bring this test to a higher degree of accuracy and help to reduce the unspecific reactions.

We wish to express our thanks to Dr. C. C. Young, Director of the Michigan State Health Department Laboratories and Dr. James E. Davis of the College of Medicine and Surgery of Detroit for their helpful support in this work.

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TENTH-NORMAL HYDROCHLORIC ACID AS A DILUENT FOR COUNTING LEUKOCYTES AFTER INFUSION OF SOLUTION OF ACACIA*

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During the World War, when solution of acacia was first used extensively, it was noticed that the leukocytes often could not be counted in the first three or four days after its infusion. More recently, Huffman[1] thought this might be caused by interference from acacia precipitated in the counting chamber. My attention was first called to the phenomenon by the laboratory technicians of The Mayo Clinic, who were able to recognize that a patient had received acacia by the appearance of the cells in the blood-counting chamber; in attempts to count the leukocytes, the microscopic field was crowded with cells.

When I attempted to count the leukocytes of a patient to whom acacia had been administered recently, using the ordinary diluent (1 per cent acetic acid), a preponderance of unhemolyzed erythrocytes was seen. This failure of hemolysis could be observed grossly, the liquid in the diluting pipette remaining cloudy. It occurred to me that perhaps a stronger acid would hemolyze the erythrocytes without destroying the leukocytes, and hence be suitable as diluent. Tenth-normal hydrochloric acid was available, and I found it apparently fulfilled these qualifications. In order to determine the accuracy of the leukocyte count when tenth-normal hydrochloric acid was used as a diluent, two counts were made on each of seventeen persons who had not been given acacia; 1 per cent acetic acid was used in one pipette and tenth-

* Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery, December, 1931. Work done in the Division of Medicine, The Mayo Clinic.

normal hydrochloric acid in another. In each case by statistical methods the count made with hydrochloric acid was as near like that made with the standard diluent as another count would have been if acetic acid had been used in the second pipettes.

TABLE 1

LEUKOCYTES, WITH DIFFERENT DILUENTS, OF PATIENTS WHO HAD BEEN GIVEN INFUSIONS OF SOLUTION OF ACACIA

CASE	WEIGHT	SOLUTION OF ACACIA INJECTED	DAYS AFTER INFUSION OF ACACIA	LEUKOCYTES IN EACH CUBIC MILLIMETER OF BLOOD, USING AS DILUENTS:	
				1 per cent acetic acid	Tenth-normal hydrochloric acid
	kgm.	*cc.*			
1	63	300	2	Indistinguishable	11,700
2	71	500	2	Indistinguishable	11,500
			4	6,800	
3	77	550	2	Indistinguishable	14,200
			5	Indistinguishable	11,800
4	74	700	2	Indistinguishable	13,200
			3	Indistinguishable	11,700
			5	7,200	
5		700	1	Indistinguishable	13,200
			2	Indistinguishable	9,900
			3	11,900	
6	77	700	1	Indistinguishable	6,200
			2	Indistinguishable	6,200
			3	Indistinguishable	7,400
			4	10,600	10,700
7		1,000	1	Indistinguishable	25,500
			2	Indistinguishable	18,400
			3	Indistinguishable	25,900
			5	15,550	13,950
8		400	2	Indistinguishable	16,700
			3	Indistinguishable	9,500
			5	Indistinguishable	7,600
			8	11,300	11,100

As the opportunity presented, the leukocytes of patients who had been given infusions of 6 per cent solution of acacia were counted; when possible, the first count was made within four hours after infusion. In some instances, the leukocytes could be counted easily with the use of either diluent; in these cases further observations were not made. If the count could not be

made by using 1 per cent acetic acid, trial counts were made each twenty-four hours by both methods. This was done in eight cases. With 1 per cent acetic acid as a diluent, the leukocytes could not usually be counted until three to five days had passed, because of the unhemolyzed erythrocytes. When the leukocytes could first be counted with this diluent, the field under the microscope still contained some unhemolyzed erythrocytes. When the diluting fluid was tenth-normal hydrochloric acid, the leukocytes could always be counted easily and satisfactorily. It was apparent grossly in the pipette that the erythrocytes were hemolyzed. On the counting slide, the leukocytes were clearly visible and erythrocytes were not seen (table 1).

It is probable that diluents other than tenth-normal hydrochloric acid might be found as useful, but I did not attempt to test other fluids. The data presented do not afford a positive explanation for the failure of 1 per cent acetic acid to hemolyze the erythrocytes of patients who had been given infusions of solution of acacia. Perhaps the greater concentration of hydrogen ions of tenth-normal hydrochloric acid (pH 1.09) as compared to 1 per cent acetic acid (pH 2.80) is a factor. Nor are these data adequate for correlating the dose of acacia, in terms of grams of acacia for each kilogram of body weight, with the length of time that the leukocytes could not be counted, using 1 per cent acetic acid. Many of the patients were critically ill during the days the leukocyte counts were made, so that the increase in some cases cannot be considered as arising from injection of solution of acacia.

SUMMARY

After infusion of solution of acacia, it is often impossible to count the leukocytes in the usual manner, because the erythrocytes are not hemolyzed by the diluent (1 per cent acetic acid). By using tenth-normal hydrochloric acid as a diluent, however, the erythrocytes are completely hemolyzed and the leukocytes can be counted accurately.

REFERENCE

(1) Huffman, L. D.: Solution of acacia and sodium chloride in hemorrhage and shock. Jour. Am. Med. Assn., **93**: 1698–1701. 1929.

AN INEXPENSIVE OCULAR RULER TO FACILITATE RETICULOCYTE COUNTING

F. M. JOHNS

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The recognition of the importance of determining the "rate" of blood production has brought a general demand upon the present day intern and student body for numerous reticulocyte counts that may be very time-consuming without the proper microscope equipment.

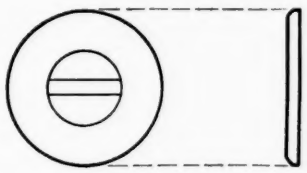

FIG. 1. CROWN GLASS DISC OF 21 MM. DIAMETER WITH TWO PARALLEL LINES 2 MM. APART

One of the chief difficulties has been the serial examination and enumeration of the stained cells in fields of the microscope containing a large number of corpuscles. Many have hesitated to request students to purchase high priced ocular micrometer discs or the still more expensive Ehrlich oculars which greatly facilitate these counts. Improvised markings on the lower lens of the ocular do not give clear-cut images and are difficult to clean off without damaging the lens.

To meet this demand I have designed a crown glass disc that fits rather loosely within the ocular casing and is supported on the ocular diaphragm. Across the center of the disc are engraved two parallel lines 2 mm. apart. In use with a thin film of corpuscles mounted under a cover glass in a mixture of brilliant

cresyl blue and sodium oxalate and viewed with the oil immersion lens a line of corpuscles is presented that may be readily counted in seriatim (see figs. 1 and 2).

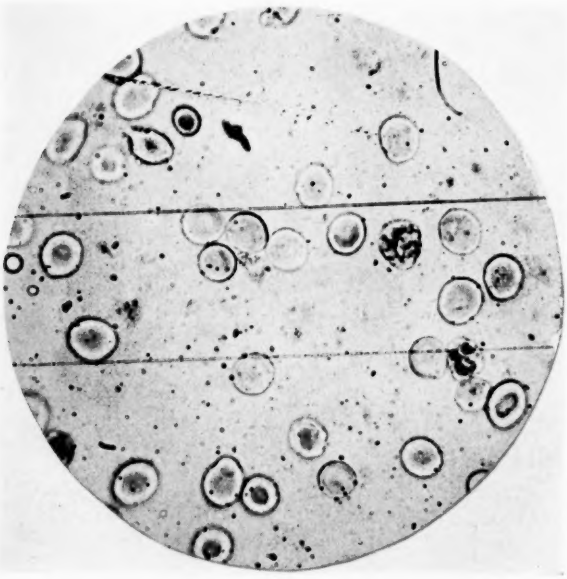

FIG. 2. THE CENTRAL PORTION OF AN OIL-IMMERSION FIELD SHOWING GUIDE LINES, ERYTHROCYTES AND RETICULOCYTES IN A FRESH PREPARATION MOUNTED UNDER A COVER GLASS

This ocular ruler has been manufactured by the Bausch and Lomb Optical Company for our medical students at a cost of about a half dollar apiece.

EDITORIAL

The Problem of Trench Mouth

Literature, especially on dentistry, abounds in papers under the headings of Vincent's disease, stomatitis, angina, trench mouth, Gilman's disease, Plaut's disease, gingivitis, ulcero-membranous stomatitis sounding like the variegated, ancient terminology of Bright's disease. Some of the thousand and one indefinite abstracts of recent years have repeatedly reiterated and popularized an acute ulcero-membranous mouth lesion due to the usually described combination, Vincent's spirillum and fusiform bacillus. Because these bacteria are considered as anaerobes, the lesions are locally treated by so-called oxygen-excess producing perborate of sodium and peroxide of hydrogen, often needless and useless intravenous arsphenamine and such escharotics as chromic and trichloracetic acids.

Recently, *Time* tersely reported the subject based on facts and fallacies of the literature in an able, popularly written review sounding in the end like a possibly inspired advertisement for the use of an expensive rather than a cheap preparation of inadequate perborate of sodium. Incidently *Time*, misinformed, advocated the intramuscular use of neosalvarsan.

The prevalent Vincent's disease or trench mouth is an acute, ulcerative, necrotic, membranous, edematous, hemorrhagic condition usually primarily involving the gingivae, less often the tonsils. The tongue, floor of the mouth, fauces, lips, sides of the cheeks, often secondarily associated are rarely primary, and when so affected, without typical gingival reaction, are due to some other specific factor.

The easily recognizable, freshly smeared spirilliform organisms, *Borrelia vincenti*, are always the long, extremely active type, in these acute cases. Their presence alone is sufficient for a diagnosis. The frequently associated fusiform bacillus, *B. fusiformis*

(Hoelling), may be present in small numbers, may play a minor rôle, may be almost absent or may appear actively motile in large numbers. The characteristic clinical odor is not produced by these organisms but by other types easily cultured on blood agar plates. Still other associated cultural organisms, streptococcus, stapyhlococcus and short bacillary forms, are often of prime importance, although usually not considered.

The disease is now reportable in several States and it has a world wide distribution. If vacationists have imported many cases from Europe, they have exported just as many and the balance of trade is equal. The greatest age incidence is young adult life, equally prevalent in boys and girls; my youngest case age six was a school infection probably a pencil borne fomes, the older cases usually follow dental extractions. Direct infection by kissing is responsible for most of the cases but fomites are always possible. Public drinking fountains where the mouth touches any part are very bad; the common communion cup is obsolete. One attack does not immunize in the strict sense nor does it increase susceptibility. The greatest danger, aside from the cases of immediate death, lies in the insidious progression to chronic suppuration with the eventual development of so-called pyorrhoea, loss of teeth and focal infection.

The disease reaches its fastigium in from five to ten days, the mouth is always very sore, eating is painful. By ceasing to brush their teeth because of pain and bleeding, the latter always very profuse, patients increase the cultural pabulum. The gingivae are intensely reddened, very boggy, bleed at the slightest touch, the whole or portions being involved. The exudate is thin and sticky, teeming with active mixed bacterial types, *Borrelia vincenti* predominating. General reactions vary from slight to high temperatures and prostration. A leukocytosis prevails but leukopenia may be found. Agranulocytosis is frequent enough to warrant taking a blood count on every case. Death as a sequela may result if after dental extractions ensuing spirilliform gingivitis and cellulitis of the neck are not controlled.

The recognition of this type of gingivitis is a responsibility of the medical man; the exact bacteriological diagnosis belongs to the laboratory, and the treatment to the dentist.

Intelligent, specific chemotherapy directed at the local condition of the mouth with general supportive hospitalization regime will easily control the situation in twenty-four hours, will have the case well in hand on the third to the fourth day, and will eliminate all evidences or traces of the disease in from ten days to two weeks. The earlier the recognition, the more prompt are the results; if the severe cases of cellulitis in the neck are not too far advanced while they will respond, they of course do so more slowly. Intelligent treatment does not include chromic acid, trichloracetic acid, perborate of sodium and arsphenamine intravenously, all of which are now commonly used. It does include arsphenamine locally, as well as the dyes of which acriviolet is a very valuable remedy in spite of a recent communication to the contrary in the *Journal of the American Medical Association*, the more recent commercial mercurials, the silver salts, Dakin's solution, at times peroxide of hydrogen and pure castile soap. The second most important factor in treatment is the method and frequency of application. Under such a regime of recognition, study and treatment acute ulcerative spirilliform gingivitis, trench mouth or Vincent's disease, is a disease in which the results are most satisfactory both to patient and physician.

ROBERT A. KEILTY.

NEWS AND NOTICES

Although each meeting of the Society seems better than the preceding one, it will be difficult for a subsequent meeting to surpass the success of the Eleventh Convention held in New Orleans from May 6 to 9.

The local committee, with Dr. F. M. Johns as chairman, left no stone unturned to have every detail worked out to furnish the maximum pleasure and profit to the attending members.

The outstanding single feature was an all day trip to the Leprosarium, where the Society was the guest of Major O. E. Denney. After a delightful dinner in the old mansion house, clinics were held and laboratory demonstrations made.

Another interesting feature was a complementary supper given by the Hotel Jung after which a roundtable discussion extended well into the night.

On Monday, May 9, the Society dedicated the new Department of Pathology of the Louisiana State University, at which time Dr. H. J. Corper and Dr. T. B. Magath made the addresses and Dr. Walter Simpson and Dr. A. G. Foord performed autopsies. Dr. F. M. Johns unveiled the dedicatory tablet.

The scientific part of the program was unusually good, especially a morning program devoted to hematology. Free and valuable discussion followed the reading of the papers.

The complete account of the business meeting will be given in a later issue of the JOURNAL.

The Ward Burdick Medal was awarded to Dr. B. S. Kline for his work on tests in syphilis and the exhibit award was made to Dr. R. R. Kracke for his exhibit on agranulocytosis.

The Scientific Exhibits included the following:

(1) DR. R. D'AUNOY AND STAFF: Pathological specimens of unusual interest and x-ray visualization of the spleen and liver following administration of thorium dioxide.

(2) DR. ROY R. KRACKE: Blood and marrow studies of agranulocytosis.

(3) Dr. B. S. Kline: The "ball" test for syphilis.

(4) Dr. F. M. Johns: (a) A pump for transfusion and other purposes utilizing the elastic recoil of the rubber tube to produce a vacuum and simultaneously forcing an almost continual flow of fluid through a system of tubes; (b) A reflex microscope for study of lesions of the crystalline lens; (c) The Johns precipitin test for syphilis, utilizing the increase in precipitating action of dried serum in the place of inactivated serum, also a combined drying and reading rack for use with this test.

The following were elected to Honorary Membership:

Col. C. F. Craig, United States
Dr. E. C. Dodds, England
Dr. E. C. Achard, France

The following were elected to membership:

Curtis, Stephen Horace
Banks, Horace McMurran
Freshman, Wm. Alexander
Lindberg, Ludwig A.
Jaisohn, Philip
Haden, Russell Landram
Thornton, H. C.
Grill, Jno. C.
Barta, E. F.
Bohls, Sidney Wm.
Stoval, Wm. Davidson
Hulsey, Simeon Hardin
Pottenger, Joseph Elbert
Maner, G. D.
Dardinski, V.

The following were elected to associate membership:

Yagle, Marguerite E.
Scherago, Morris

The following officers were elected:

President-Elect: Dr. A. G. Foord
Vice-President: Dr. F. M. Johns
Members of the Executive Committee: Dr. H. J. Corper
Dr. C. I. Owens
Members of the Board of Censors: Dr. F. H. Lamb
Dr. W. S. Thomas
Members of the Board of Registry: Dr. Philip Hillkowitz
Dr. Kano Ikeda

BOOK REVIEWS

Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis. By B. S. Kline. Pp. viii + 99, 1932, Baltimore, The Williams & Wilkins Company, $2.50.

In the last few years there has been a tremendous development in precipitation and flocculation tests for syphilis. Dr. Kline has been one of the foremost contributors to this subject and in the present volume brings together the results of his researches up to the present time. While this book is primarily intended for the individuals who are going to actually perform these tests, it will prove valuable to the physician who is interested in syphilis, since it contains important information relative to the evaluation of such laboratory procedures According to the author the slide precipitation test differs from flocculation tests in general in that (1) the antigen is the acetone insoluble fraction only of alcoholic heart extract, (2) the antigen is more stable and more uniformly sensitive than those in general use, (3) the reactions are carried out on optimal open polished surfaces of microscopic slides in cells of optimal proportions and the results are read accurately with the aid of a microscope.

The book gives in detail, and with many illustrations, the exact method of performing the Kline test; both the diagnostic and exclusion slide tests with heated and unheated serums and with spinal fluids. A section of the book is devoted to a clinical consideration of the test and the author indicates that this test is 10 per cent more sensitive than the Wassermann reaction.

A preliminary report is made of the "ball" test which is a tube test using the centrifuge to cause the development of the ball. There is an excellent bibliography at the close of the volume. This small book will find great use in the laboratory since it brings together in a few pages the things necessary to know about these tests.

Biochemistry in Internal Medicine. By MAX TRUMPER AND ABRAHAM CANTAROW. Pp. 454, 1932, Philadelphia, W. B. Saunders Company, $5.50.

This book is primarily intended for clinicians and is an attempt to bring together the modern advances in physiology and biochemistry as encountered daily in internal medicine. The authors have made no attempt to make a laboratory manual out of the volume and give only a few technical methods. While the authors refer to various contributors to this field, only nine references are given in the text which will make the book of little value to the critical student. The book will give to the clinician a general insight into the application of some of the new discoveries in physiology and chemistry as applied to every day medicine. Much of the material contained in this book can be found in general laboratory manuals but the authors have, by leaving out the technical details, made the material more readable. The book will prove of most value to the clinician who does not have the time or the training to acquaint himself with the details of scientific advances in medicine, but does have time to read a short summary of the outstanding contributions to physiology and chemistry as related to specific diseases.

In accordance with the plan of the JOURNAL to call attention to our advertisers, it is suggested that you make especial note of the advertisement by The Empire Laboratory Supply Company, a well known firm which has advertised in the JOURNAL since the first issue. This firm introduced into America the Seitz Germicide Filters which have found a favorite place in American bacteriological laboratories due to their simplicity, efficiency and low operating cost.